PARASITOLOGY

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OBSERVATIONS ON *BLASTOCYSTIS HOMINIS.*

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(With Plates XVI, XVII.)

1. *Introduction.*

In 1908 intestinal parasites of man were described by Bohne and Prowazek. The authors considered them to be cysts of *Trichomonas hominis*. The parasites consist of a highly refractile spherical body[1], staining yellow or yellowish brown with iodine and pale grey or black with iron haematoxylin; this sphere is surrounded by a narrow plasmatic layer, containing the nuclei. Bensen (1910) gave a detailed description of these bodies, with stages of autogamy, and considered them also to be cysts of *Trichomonas*. Prowazek (1911) did not subsequently change his opinion regarding the systematic position of these bodies and James (1914) confirmed Prowazek's views by stating that cats infected with the same bodies showed *Trichomonas* in their stools.

Wenyon (1910) was the first to contest Prowazek's views in holding the opinion that these bodies are degenerative stages of *Chilomastix mesnili*. Alexeieff (1911), who saw them in the gut of *Haemopis sanguisuga*, considered them to belong to a new genus and species (*Blastocystis enterocola*), showing no affinities with *Trichomonas* or any other flagellate but being related to the Blastomycetes. This opinion is founded on the following facts: The parasite has a mucous membrane; it multiplies by budding; four to eight spores are formed within its body like ascospores in an ascus, they have a germinative pore; the structure of the nucleus (peripheral chromatic granule, separated from

[1] In this paper referred to by the name of "the sphere."

the nucleoplasm by a clear space) resembles that of Blastomycetes. Subsequent authors confirm Alexeieff's views by stating that the parasite is a Blastomycete or a chytridiaceous fungus, without however describing anything like the sporulation as seen by Alexeieff. Brumpt (1912) describes the nuclei as containing a central large karyosome, differing in this respect from Alexeieff. Chatton and Lalung Bonaire (1912) observed the sphere to be eosinophilous. Mathis (1913) and Low (1916) also consider these parasites to be of a separate species. Scott Macfie (1915), although of the same opinion, thinks that it is related to intestinal amoebae. Alexeieff believes that the human *Blastocystis* and that found in the intestine of apes and rats all belong to the species *B. enterocola*, but Brumpt places these forms in different species. Alexeieff also thinks that the parasite is related to *Dermocystis pusula* Pérez (1907), a skin parasite of *Molge marmorata*, showing a refractile sphere similar to that of *Blastocystis*.

I have often observed *Blastocystis* in the stools of Europeans, having resided in the Dutch East Indies. Owing to the refractile sphere these bodies, in eosin preparations[1], are often taken for cysts, and so might cause error in diagnosis. Moreover Low points out that only after gaining a more thorough knowledge of the life cycle of this parasite will it be possible to know whether it is pathogenic or not. Therefore it seemed to me to be of importance to investigate the life history of the human *Blastocystis*.

2. *Observations on fresh material.*

In fresh material, *Blastocystis* appears as a hyaline rounded body surrounded by a less refractile fringe. The latter takes the shape of a ring or of two or three crescents. After some minutes' observation under the cover-glass, the sphere is seen to lose its refringence and to disappear, leaving only a vacuole surrounded by less refractile substance. Treated by iodine or Lugol's solution, the fringe stains yellow, the sphere remains unstained. In 5 % formalin the sphere disappears instantly and the fringe becomes highly refractile. In an eosin solution the fringe generally stains red, but sometimes it is colourless and only stains after being kept for some time under the cover-glass. I therefore conclude that this fringe constitutes a protoplasmic layer

[1] An eosin preparation consists of a suspension, under the cover-glass, of fresh fecal matter. All living elements (amoebae, cysts, etc.) remain unstained and are seen as white patches on a red background; all dead elements are stained by the eosin (cf. Kuenen and Swellengrebel, 1913).

and that it only seldom can be observed in a living condition. The sphere never stains with eosin. I cannot confirm Chatton and Lalung Bonaire's view on this subject. After being coloured by eosin, dark staining granules may be observed in the plasmatic fringe, no cyst-wall was observed to surround the cell, consequently *Blastocystis* is not a cyst.

With the object of determining whether *Blastocystis* is a normal developmental form of *Trichomonas* or *Chilomastix* the following experiments were undertaken:

Case No. 1. European, having resided in the Dutch Indies for some years, returned October 1913. The stools contain *Limax* amoebae, *Entamoeba coli* and *Blastocystis*, they were regularly observed for several months, but no other parasites were found. After the administration of four cascara tabloids, many blastocysts were present in the semi-fluid stools; after strong purgation (30 grams of sulphate of magnesia) the stools became fluid but no flagellates of any kind were found. A diet consisting of milk and some eggs only caused the blastocysts to diminish in number but not to disappear.

Case No. 2. Javanese, temporarily residing in Europe. In the stools *Limax* amoebae and *Blastocystis* were present, and observation of three months' duration did not reveal any other parasites. Purgation with sulphate of magnesia (15 grams) did not cause the appearance of flagellates.

These experiments show that *Blastocystis* may be continually present in the intestine without any traces of flagellates being found even after prolonged observation. Although in other cases I found *Blastocystis* together with *Chilomastix mesnili*, the former observations show that even if blastocysts can be formed from *Chilomastix* they also may be formed without the help of this flagellate.

3. *Observation on fixed and stained preparations.*

Wet-fixed (corrosive alcohol) preparations were stained with iron- or Delafield's haematoxylin. The results obtained from these observations will be recorded for each case separately:

Case No. 2 (Pl. XVI, Figs. 1–27).

(*For particulars of this case see above.*)

With iron-haematoxylin, the sphere stained yellowish-grey (Figs. 14–19). The surrounding cytoplasm stained a pale grey, it showed an alveolar structure (Figs. 14, 15, 17) or no structure at all (Fig. 16),

sometimes it consisted of nothing but a line around the sphere (Figs. 19, 20). No trace could be found of a cyst-wall, pellicula, or mucous membrane, not even in specimens showing shrinkage. The chromatic granules were generally found in the plasmatic fringe (Figs. 14, 17), sometimes within the sphere (Fig. 15). There may be one chromatic body, surrounded by a clear zone (Fig. 14), sometimes showing a granule in its centre (Fig. 16), or there may be two or more of them of equal or unequal (Fig. 23) dimensions, some of them showing an internal structure (Fig. 27). The sphere may be hyaline and without any internal structure (Figs. 14–18) or small alveolae may be present (Figs. 13, 20, 24, 27). Sometimes it stains uniformly yellowish-grey with iron-haematoxylin, or deep black (Fig. 25), sometimes the centre stains black and the periphery yellow-grey (Fig. 27).

In the stools of this case all blastocysts had their cytoplasm stained red after treatment with eosin, which shows that none were found living. Consequently all the forms mentioned here must show more or less marked necrotic changes. Which forms show the least deformity? I believe that the least altered forms are those possessing the broadest cytoplasmatic fringe because in Case No. 3, the only one in which still living blastocysts were seen, most of them showed a broad cytoplasmatic layer. In Case No. 2, most of the blastocysts only showed a very narrow cytoplasmatic layer (Figs. 19 and 20), therefore I believe that such forms should be considered as the final stages of necrosis, although some persons might be tempted to consider them as representing stages of sporulation. The problem now arises as to the origin of the stages with broad cytoplasm (Figs. 13–17).

Sometimes blastocysts were observed with a chromatic reticulum within the sphere (Fig. 12) nearly (Fig. 11) or wholly (Figs. 9, 10) filling the latter. Apparently there is a genetic relationship between these forms, but what is the first stage? Here Figs. 5–7 may suggest a solution. Fig. 5 represents an amoeba of the *Limax* type, showing within its cytoplasm a dark staining part of reticular structure surrounded by a pale staining part of alveolar structure. In Figs. 6 and 7 this differentiation is more distinctly marked and these forms are not to be distinguished from those represented by Fig. 10. In Fig. 9 there is a distinct demarcation between the two plasmatic components, also shown by differences in colour, the peripheral crescent-shaped cytoplasm being coloured grey and the alveolae between the chromatic reticulum of the centre being coloured yellow-grey. A similar differentiation is shown in Fig. 8, but here the central cytoplasm does not

show a reticular structure and the nucleus has assumed the shape of a crescent, a deformity often found in degenerating amoebae of this kind (cf. Fig. 1).

Another way in which *Blastocystis* may arise from *Limax* amoebae is represented in Figs. 1–4. The centre of the cytoplasm shows large vacuoles (Fig. 1) which become fused and produce one large vacuole (Fig. 2) filled with a grey granular or reticular substance. The nucleus breaks up in chromatic granules (Figs. 3, 4). Similar changes were observed in case No. 5 (see below).

Case No. 1 (Pls. XVI, XVII, Figs. 28–64).

(*For particulars regarding this case see p.* 453.)

The smaller forms perfectly resembled the blastocysts of the former case. A peculiarity of this case was the presence of large blastocysts, their size being due to the growth of the sphere accompanied by a reduction in the volume of the plasmatic fringe (Figs. 56, 57) and of the number of chromatic granules. Within the plasmatic fringe a peculiar differentiation was to be observed, consisting of the splitting up of this fringe in an outer filamentous often contorted part (Fig. 54) and an inner plasmatic part. Fig. 56 shows the beginning of this differentiation. The inner part disappears completely, finally leaving nothing but the sphere surrounded by the filamentous part (Fig. 58).

Divisional stages, mentioned by various authors, were often met with in this case (No. 1), showing all stages of division (Figs. 59–64). It was noticed that division was more often seen in the blastocysts showing the filamentous deformation of the cytoplasm mentioned above, which is accompanied by the complete disappearance of the cytoplasm. Consequently I think that this division is due to an unbalanced condition arising from the hypertrophy of the sphere combined with a gradual disappearance of the enclosing cytoplasm, which causes the sphere to break up into two or more parts (Figs. 62, 64), which are kept together by the surrounding cytoplasm.

As in Case 2, the blastocysts of Case 1 could be referred to *Limax* amoebae (Fig. 28) showing within their cytoplasm a more or less compact agglomeration of chromatic substance (Figs. 29–31). This agglomeration grows (Figs. 32–34) and gradually (beginning at the periphery) is converted into a structureless hyaline mass (Figs. 35–38). The nucleus is often situated within the latter mass and generally disappears, being replaced by chromatic granules at the periphery which can sometimes

be shown to arise from the nucleus. No signs of sporulation were found in this case.

Case No. 3 (Pl. XVII, Figs. 65–82).

A European suffering from amoebic dysentery.

Infection with *Blastocystis* combined with *Chilomastix mesnili* and *Entamoeba histolytica*.

In this case for the first time living blastocysts (not staining red with eosin) were encountered. The sphere was generally much smaller in relation to the surrounding cytoplasm, than in the former cases (Figs. 70–75). The blastocysts differed also from the former by the presence of a vesicular nucleus, with only small chromatic granules and a distinct reticular nucleoplasm (Figs. 71–73); in other forms only peripheral chromatic granules were to be seen (Figs. 75–77). Division was observed (Fig. 81), but only combined with the filamentous differentiation of the cytoplasm already mentioned (Case No. 1).

In Case No. 3 it was quite clear that the blastocysts were formed by *Chilomastix mesnili* (Fig. 65), which often showed near the nucleus a greyish coloured body (Figs. 66, 67) which by growing fills up the greater part of the cell (Figs. 68, 69), thus producing forms identical in appearance with the blastocysts shown in Figs. 71–73.

Case No. 4 (Pl. XVII, Figs. 83–95).

Rat infected with *Chilomastix* and *Blastocystis*. Preparations kindly furnished by Dr S. L. Brug.

The blastocysts in this case were characterised by the distinct vacuolar structure of the cytoplasmatic fringe. Divisional forms were common (Fig. 89) without however showing the peculiar changes, described in the former cases. The nucleus, when present, showed the structure described in Case No. 3 (Figs. 84, 85, 86, 93). Besides these nuclei, chromatic granules were present. Sometimes the peripheral cytoplasm was reduced to a line (Fig. 95); other forms might suggest sporulation (Fig. 92) but no other stages were found supporting this view.

As in the former case, the blastocysts were formed by the rounded forms of *Chilomastix* (Fig. 83) which accompanied the flagellate stages not figured here. These forms show a strongly vacuolated cytoplasm; between the vacuoles a grey structureless substance appears (Fig. 83) which by growing pushes the vacuoles to the periphery (Fig. 84), takes

a central position (Fig. 85) and finally assumes a distinct shape with well-marked outlines (Fig. 86).

Case No. 5 (Pl. XVII, Figs. 96–106).

European child having resided in India for several years. Infection of *Blastocystis* and *Limax* amoebae.

The blastocysts of this case can be referred to forms illustrated in Fig. 96 representing small *Limax* amoebae showing a differentiation of their cytoplasm into a dense peripheral zone and a central space of loose reticular structure. The vacuoles of this part fuse into one central vacuole (Figs. 97, 98), containing several irregular chromatic particles which probably arise from the hypertrophying nucleus (Figs. 98, 100). Divisional forms occur and also stages (such as are represented in Fig. 104) resembling sporulating stages as described by Alexeieff, but no other signs of this phenomenon were encountered.

Conclusions.

1. In two cases *Blastocystis* was found where the presence of *Trichomonas* or *Chilomastix* could be excluded with absolute certainty. Consequently *Blastocystis* cannot be a normal developmental form of either.

2. In fresh stools *Blastocystis* is but seldom found to be alive and even when encountered in this state it dies quickly. After death the central sphere soon disappears.

3. The size of blastocysts varies greatly and the larger they grow the smaller becomes the peripheral fringe of cytoplasm. Living blastocysts are relatively small and rich in cytoplasm.

4. The blastocysts of the cases mentioned here, although having some general characters in common, differed much as to details of structure. This difference was especially marked when the associated parasites were different. No blastocysts were found without an associated parasite.

5. The occurrence of blastocysts in the stools of a man fed on milk and eggs only, and the presence of living blastocysts in the man's stools, exclude the idea of their being remains of solid food.

6. It is probable from the observation recorded in this paper, that "*Blastocystis*" is not the name of a zoological genus but of a peculiar form of degeneration to which representatives of different genera of intestinal protozoa may be liable. The resemblance seen in blastocysts

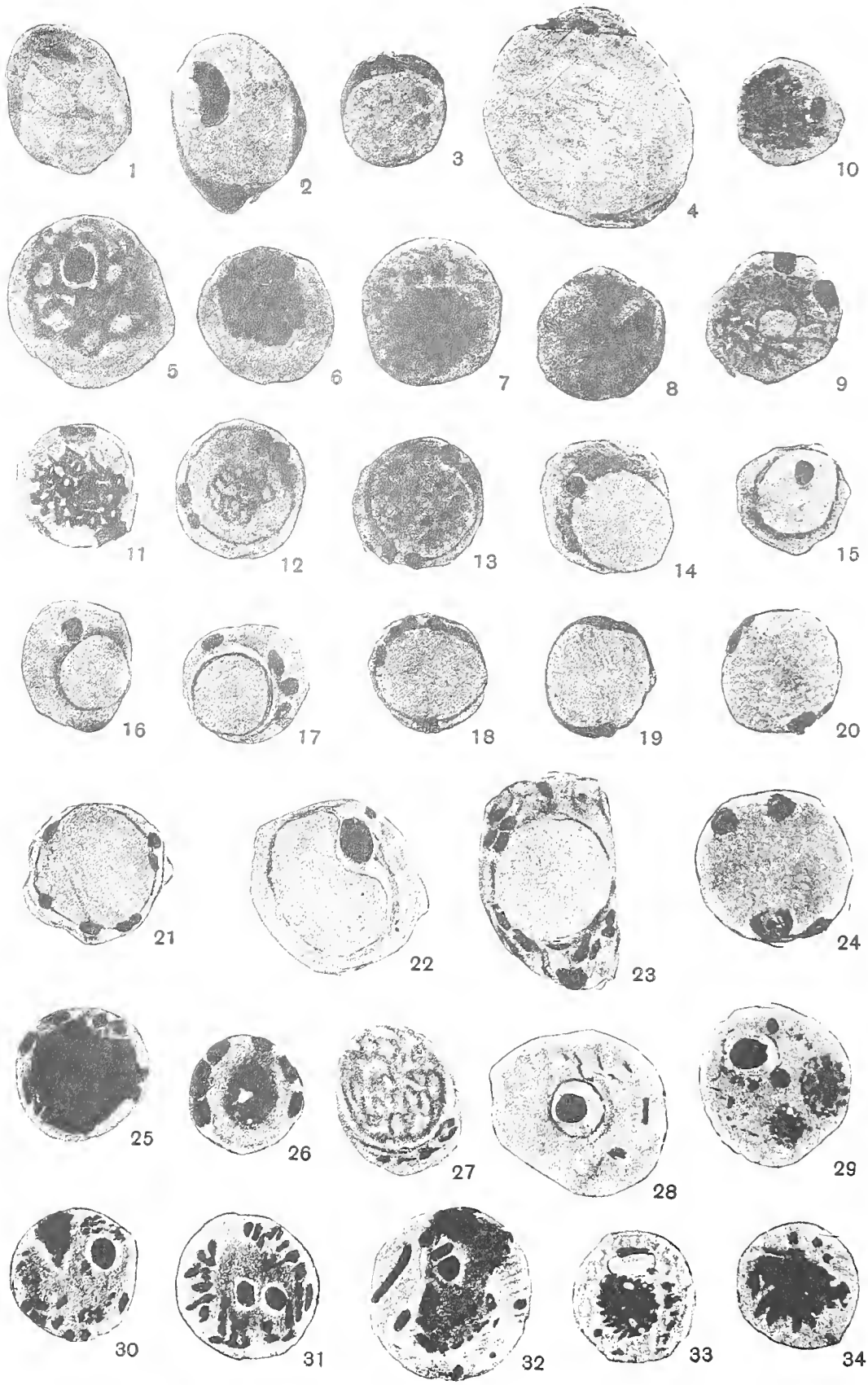
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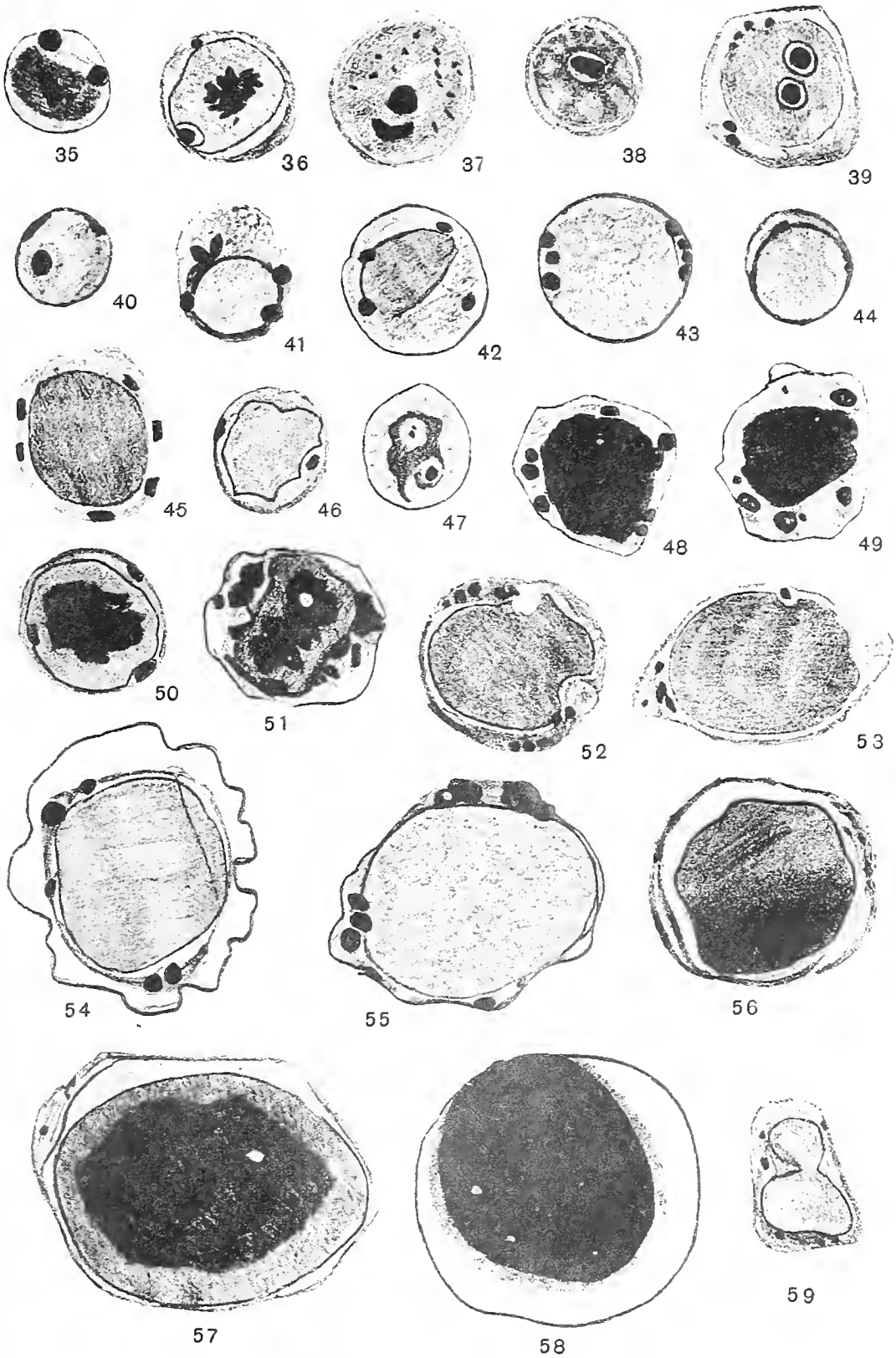
PLATE XVI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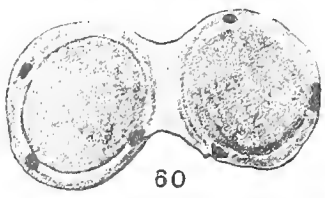
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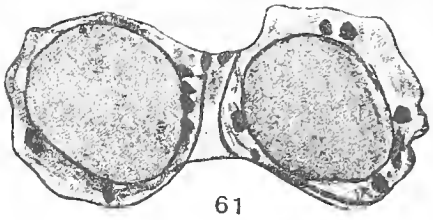
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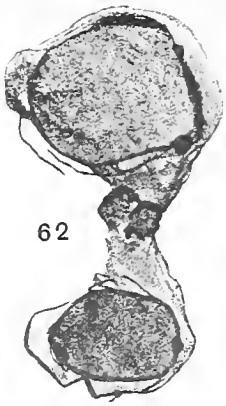
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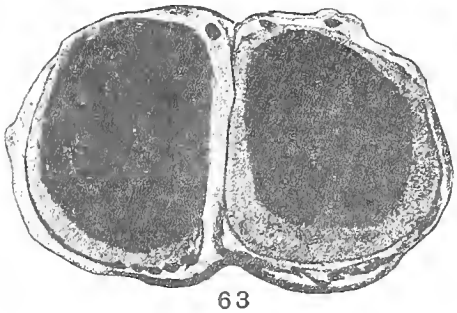
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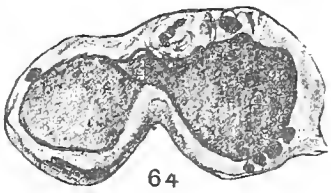
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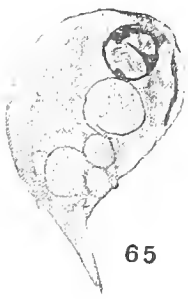
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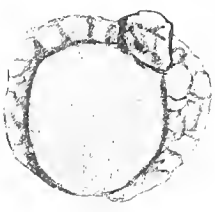
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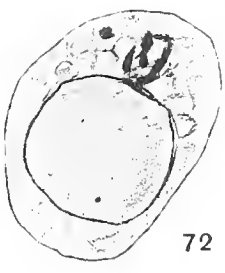
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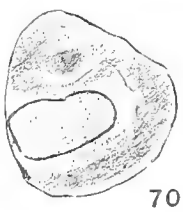
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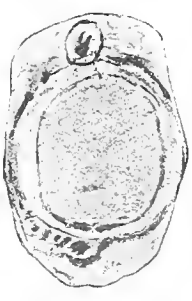
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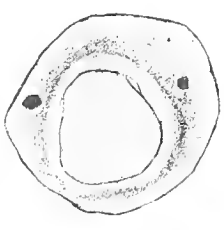
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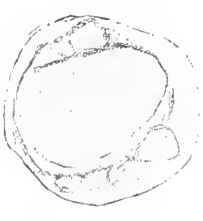
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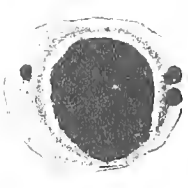
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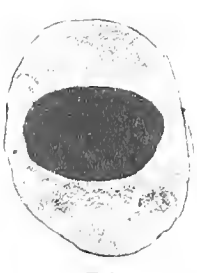
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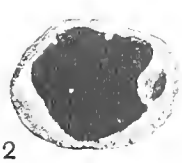
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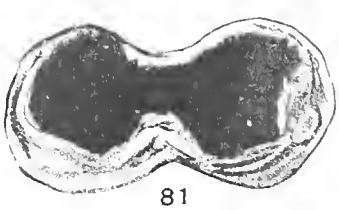
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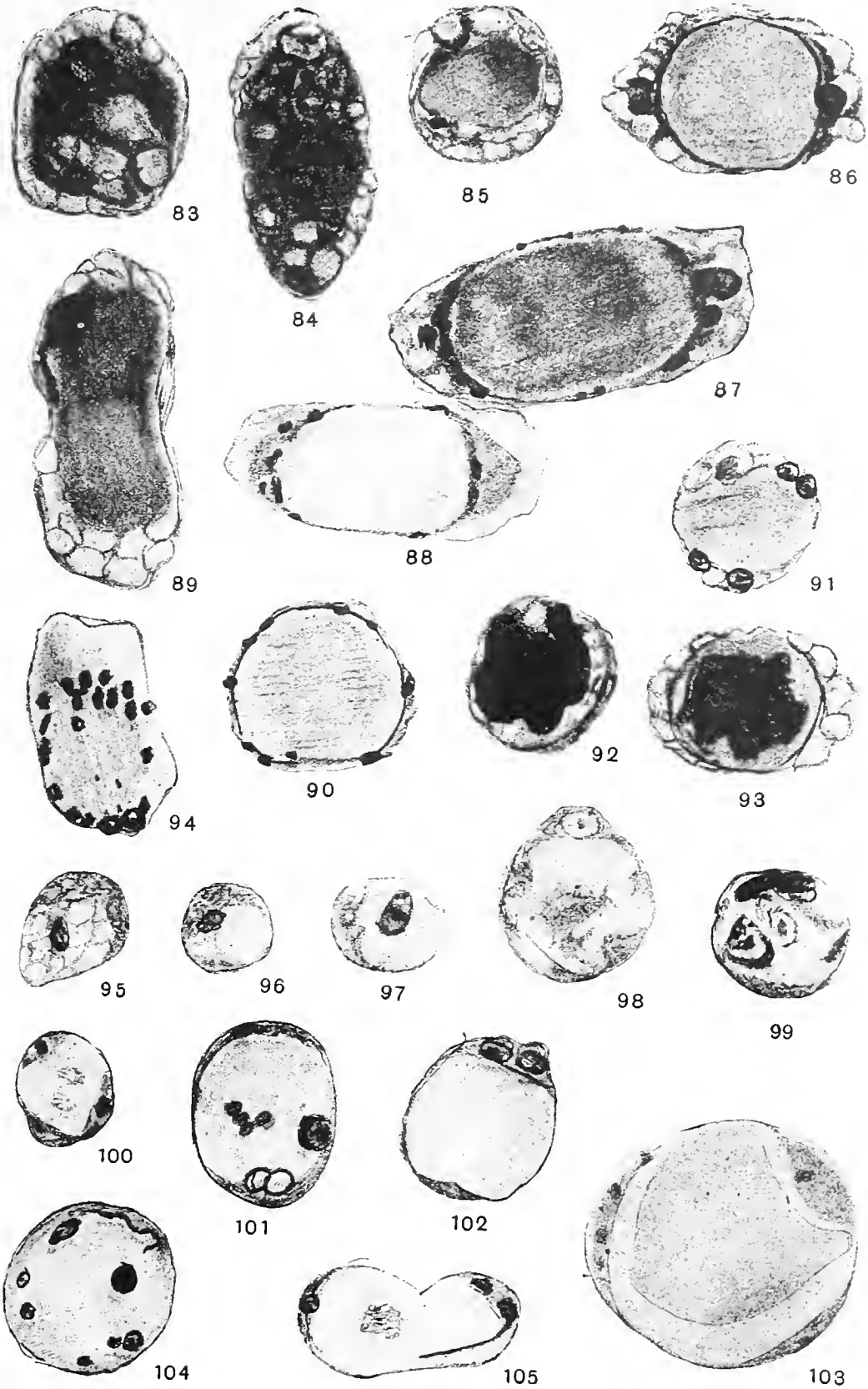
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from different sources which may lead to their being regarded as belonging to one species, is easily explained by a convergence resulting from the parasites which produce the blastocysts losing their characteristics during the process of degeneration.

7. No certain stages of sporulation were seen, as described by Alexeieff, and the nuclear structure, although variable, never resembled that given in his description. It is therefore probable that Alexeieff's *Blastocystis enterocola* is different from the forms described in man under the same name.

EXPLANATION OF PLATES XVI AND XVII

Figs. 1–27. *Case No.* 2.

Figs. 1–4. Production of blastocysts from *Limax* amoebae by the formation of a central vacuole.

Figs. 5–13. The same, by the formation of a central chromatic reticulum.

Figs. 14–17; 22, 23. Blastocysts with well-developed cytoplasm.

Figs. 18–21. Linear cytoplasm.

Fig. 24. Stage suggesting sporulation.

Figs. 25, 26. Dark staining sphere.

Fig. 27. Sphere with alveolar structure.

Figs. 28–64. *Case No.* 1.

Figs. 28–39. Production of blastocysts from *Limax* amoebae by the formation of a central chromatic reticulum.

Figs. 41, 42. Well-developed cytoplasm.

Figs. 40, 43, 45, 52, 53. Linear cytoplasm.

Figs. 46, 47. Gradual disappearance of the sphere.

Figs. 48–51. Dark staining sphere.

Figs. 54–58. Large blastocysts showing disappearance of cytoplasm.

Figs. 59–64. Division.

Figs. 65–82. *Case No.* 3.

Figs. 65–69. Production of blastocysts from *Chilomastix*.

Figs. 70–73. Vesicular nucleus.

Fig. 74. Disappearance of the nucleus.

Figs. 75–77. Involution of the cytoplasm.

Fig. 78. Dark staining sphere appearing in rounded *Chilomastix*.

Figs. 79, 80, 82. Blastocysts with dark staining sphere.

Fig. 81. Division.

Figs. 83–94. *Case No.* 4.

Figs. 83–85. Production of blastocysts from rounded forms of *Chilomastix*.

Figs. 86, 87. Forms with vesicular nuclei.

Fig. 88. Only chromatic granules present.

Fig. 89. Division.

Figs. 90, 94. Linear cytoplasm.

Fig. 91. Form suggesting sporulation.

Figs. 92, 93. Dark staining sphere.

Figs. 95–105. *Case No.* 5.

Figs. 95–98. Production of blastocysts from *Limax* amoebae by formation of a central vacuole.

Figs. 99–101, 104. Dark staining patches in the sphere.

Fig. 102. Linear cytoplasm.

Fig. 103. Form resembling a stage of sporulation as described by Alexeieff.

Fig. 105. Division.

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SUR QUELQUES NÉMATODES DES OISEAUX DE LA RUSSIE.

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(Avec les Planches XVIII et XIX et une figure dans le texte.)

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Le présent ouvrage est consacré à la caractéristique de quelques espèces de Nématodes d'oiseaux, trouvés sur le territoire de l'Empire de Russie. La plupart des formes décrites ici m'ont été gracieusement données à étudier par le Professeur de l'Académie Impériale Militaire de Médecine N. A. Cholodkowski, et je saisis l'occasion de lui exprimer ma plus sincère gratitude.

A part la description de deux nouvelles espèces (*Habronema seurati* et *Diplotriaena bargusinica*) j'établis ici une nouvelle sub-famille *Diplotriaeninae* nov. sub-f. pour trois genres de filaires d'oiseaux *Diplotriaenae* R. et H. 1909, *Serratospiculum* mihi 1915, et *Contortospiculum* Skrj. 1915. Ensuite je donne la description des mâles *Aprocta turgida* St. que la littérature ne possède pas encore et j'indique quelques nouveaux détails sur la structure de plusieurs autres nématodes.

A. Fam. *ACUARIIDAE* Seurat 1913.

I. Gen. *Acuaria* Bremser 1811.

1. *Acuaria* (*Synhimanthus*) *brevicaudata* Duj. 1845.

Littérature. DUJARDIN (1845). *Hist. nat. d. Helminth.* Paris, p. 85.—— MOLIN (1860). *Sitzungsbericht d. k. Akad. Wissensch. Wien,* XXXIX. 498.—— WALTER (1866). *Ber. Verein f. Naturkunde Offenbach,* VII. 23.——DIESING (1851). *Systema Helminth.* II. 231. *Histiocephalus brevicaudatus.*——STOSSICH (1891). Il Genere *Dispharagus* Duj. *Boll. d. Soc. Adriatica di Sc. Natur. in Trieste,* XIII. 81, No. 27. *Dispharagus brevicaudatus* Duj. (à la catégorie Spec. inquirende).——RAILLIET, HENRY et SISOFF (14. xii. 1912). Sur les affinités des Dispharages [*Acuaria* Bremser 1811]. *C. Rend. Soc. Biologie,* LXXIII. 622 (assimilation du parasite au genre *Acuaria* Brems. sous genre *Synhimanthus*).

Pl. XVIII, figs. 1, 2.

Hôtes du parasite: *Botaurus stellaris* L.; *Ardetta minuta* L.

Localisation: sous la cuticule du gésier.

Distribution géographique: Europe (France, Russie).

Ce parasite a été décrit d'une manière extrêmement précise par Dujardin en 1845; il est très à regretter qu'il n'en ait pas donné l'illustration; après Dujardin cette espèce a été étudiée par Walter en 1866, mais d'un autre hôte.

Jusqu'à présent notre connaissance de l'*Acuaria brevicaudata* est restreinte à ces deux descriptions.

Dans un des flacons avec des nématodes qui m'a été donné par le prôfesseur N. A. Cholodkowski, j'ai réussi à découvrir des femelles de cette intéressante espèce, trouvées dans le gésier du *Botaurus stellaris* en Russie.

Vu que jusqu'à présent la littérature ne possède pas encore l'illustration de ce parasite, je donne dans le présent ouvrage les figures de la partie céphalique et de la partie postérieure de la femelle, faites avec toute la minutie possible à l'aide de l'appâreil à dessiner de Zeiss, de même que sa description détaillée.

La longueur du corps des exemplaires que j'ai étudiés variait entre 7·5–8·5 mm. ; la largeur maximale était 0·29 mm. (Les femelles obtenues par Dujardin atteignaient 11·6 mm., ayant une largeur de 0·29 mm.) La partie antérieure du corps est attenuée ; la largeur du corps dans la région de l'œsophage musculaire atteint 0·17 mm. Le bout céphalique est pourvu de deux lèvres coniques et saillantes qui bornent l'orifice buccal. Ce dernier mène à un pharynx allongé et extrêmement mince qui atteint une longueur de 0·21 mm. Le pharynx passe dans la partie musculaire de l'œsophage, considérablement plus large, atteignant 0·85 mm. de longueur. Je n'ai pas pu, à mon grand regret, examiner la longueur de l'œsophage glandulaire, car tout le corps du parasite était rempli d'œufs. Le matériel étant mal conservé il n'a pas été possible de préciser l'anneau nerveux. Les cordons cervicaux sont particulièrement intéressants. Commençant à la base des lèvres, ces cordons descendent et ayant franchi 0·425 mm. ils se redressent et se réunissant deux par deux, ils forment un arc fermé à une distance de 0·23 mm. du bout céphalique. La structure de ces cordons est suivante : à mesure de leur éloignement du bout céphalique ils s'élargissent graduellement, comme l'a déjà justement remarqué Dujardin, et leur largeur atteint son apogée à la jonction ascendante. Puis chaque cordon semble être composé de trois parties.

1. *La partie intérieure*, la plus large, est transversalement rayée (ces rayures cependant ne sont pas droites, mais ondulées, comme c'est indiqué sur mon dessin).
2. *La partie mitoyenne* est étroite et lisse.
3. *La partie extérieure*, très étroite et également transversalement rayée. La largeur de la partie mitoyenne et de la partie extérieure du cordon est constante sur toute son étendue ; la partie intérieure au contraire s'élargit dans la direction de la tête à la jonction ascendante.

A une distance de 0·56 mm. du bout céphalique est disposée une papille cervicale de forme tridentée. Le bout postérieur est pourvu d'un appendice conique avec une sommité arrondie, à la base duquel se trouve l'anus (à une distance de 0·068 mm. du bout de la queue).

La vulve est disposée à une distance de 0·6 mm. de l'extrémité postérieure. Les œufs de forme arrondie ont jusqu'à 0·03 mm. de longueur et une largeur de 0·025 mm.

A mon grand regret je n'ai pas eu à ma disposition de mâle de cette espèce.

B. Fam. *SPIRURIDAE* Railliet et Henry 1911.

II. Gen. *Habronema* Dies. 1861.

Sous l'enveloppe cuticulaire du gésier d'oiseau se localise un grand nombre d'espèces de Nématodes, la plupart desquels doivent être attribués aux ordres *Spirurata*, *Strongylata* et *Trichurata*. Parmi ces parasites une place importante est occupée par les représentants du genre *Habronema* Dies. (de la famille *Spiruridae* Railliet et Henry 1911), trois espèces duquel, *H. leptoptera* Rud., *H. unilateralis* Molin et *H. mansioni* Seurat 1914, habitent le gésier des oiseaux de proie.

Parmi les nématodes de la collection du Cabinet Zoologique de l'Académie Impériale Militaire de Médecine, que le prof. N. A. Cholodkowski m'a aimablement remise pour déterminer, j'ai découvert une nouvelle espèce du genre *Habronema* Dies. du gésier d'un *Falco cenchris* tué en 1897 à l'Altaï (préparation No. 46). J'appellerai cette espèce *Habronema seurati* n. sp. en l'honneur de l'éminent hélminthologiste L. Seurat d'Algers.

2. *Habronema seurati* nov. spec.

Pl. XVIII, figs. 3—7.

Hôte: *Falco cenchris.*

Localisation: gésier.

Distribution géographique: Altaï (Sibéric Occidentale). Ce type se trouve au Cabinet Zoologique de l'Académie Impériale Militaire de Médecine.

Description de l'espèce:

De petits Nématodes, dont le corps cylindrique est atténué vers les deux extrémités, surtout vers l'extrémité antérieure. La cuticule assez puissante est transversalement striée; la distance entre les striures transversales contiguës atteint 0·0145 mm. En examinant attentivement à un grossissement considérable les striures transversales on voit que le bord libre postérieur de chaque anneau transversal a une apparence festonnée (voir Pl. XVIII, fig. 4).

Les ailes latérales, très faiblement développées, commencent un peu en arrière du niveau des papilles cervicales. Ces dernières se trouvent en avant de l'anneau nerveux (chez le mâle) à 0·144 mm. de l'extrémité céphalique; grâce à leur dimension minuscule il est difficile de les distinguer. L'anneau nerveux se localise un peu en avant de la mi-longueur de la partie musculaire de l'œsophage (chez

le mâle à une distance de 0·255 mm. de l'extrémité céphalique). La cavité buccale est limitée par quatre lèvres : deux lèvres latérales portant une dent à la face interne, et deux lèvres, l'une dorsale l'autre ventrale, portant à la partie mitoyenne une petite dent acuminée. En général les lèvres rappellent beaucoup par leur structure celles de l'espèce *Habronema mansioni* Seurat.

Le *mâle* atteint 5·5 mm. de longueur sur une épaisseur maximum de 0·3 mm. Au niveau de l'endroit où la partie musculaire de l'œsophage passe vers la partie glandulaire, l'épaisseur du corps atteint 0·136 mm., et 0·27 mm. dans la région de la partie terminale de l'œsophage. Le fond de la capsule buccale est à 0·042 mm. de l'extrémité céphalique ; la capsule buccale est large de 0·011 mm. La partie musculaire de l'œsophage atteint 0·425 mm., la partie glandulaire (postérieure), 2·77 mm. L'anneau nerveux se trouve à une distance de 0·255 mm. de l'extrémité de la tête. L'extrémité caudale est pourvue d'une bourse allongée dont les ailes (du côté ventral) présentent des striures longitudinales. Les striures détachées ont l'air de lignes ondulées. La partie ventrale de l'extrémité caudale est ornée d'une sculpture spéciale : toute la cuticule de cette partie du corps, jusqu'au niveau de l'orifice cloacal, est caractérisée par des striures ou plis longitudinaux, d'une forme très caractéristique (Pl. XVIII, fig. 5). L'épaisseur de chaque pli atteint 0·011–0·013 mm. Le bord libre de ces plis proéminents a des dentelures, qui sont régulièrement distribuées. Immédiatement en arrière du cloaque la cuticule (sauf les ailes) devient lisse et seulement après la première paire de papilles postanales pédonculées, on voit de nouveau trois rangs de petits boucliers.

La cuticule de l'extrémité de la queue est de nouveau lisse.

Les deux spicules sont de forme et de grandeur inégale. Le spicule droit, court, atteint 0·45 mm. de longueur et a la forme d'une tige épaissie à la base avec une extrémité postérieure recourbée. Cette dernière rappelle par son aspect la petite cuiller acérée du chirurgien (Pl. XVIII, fig. 7). Le spicule gauche (fig. 6), extrêmement mince et long, atteint 2·1 mm. de longueur. Sa partie postérieure a la forme d'un croc avec un surgeon postérieur pointu et lancéolé et avec un petit surgeon latéral légèrement courbé ; le surgeon latéral n'atteint que 0·006 mm. de longueur. Le petit gubernaculum est caractérisé par un surgeon dirigé du côté gauche. Les papilles caudales chez le mâle sont de deux types : des papilles pédonculées (quatre paires préanales et deux paires post-anales), et des petites papilles sans pédoncules, disposées au sommet de l'extrémité caudale (9–10 pièces).

A part cela sur la lèvre antérieure du cloaque se trouve une papille médiane impaire.

Le cloaque se trouve à une distance de 0·2 mm. de l'extrémité caudale.

La *femelle* atteint 9·2 mm. de longueur sur une épaisseur maximum de 0·37 mm. La capsule buccale a une longueur de 0·034 mm. sur une épaisseur de 0·017 mm. L'extrémité caudale est de forme conique avec un petit surgeon obtus. La vulve est disposée à 3·4 mm. de l'extrémité caudale (dans la partie postérieure du corps). Les œufs ont jusqu'à 0·047 mm. de longueur sur une épaisseur de 0·0275 mm.

En comparant ce nouveau parasite d'oiseaux de proie avec d'autres espèces dont il est parent, nous pouvons conclure qu'il a la plus grande ressemblance avec les *Habronema mansioni* Seurat desquels il se distingue cependant par les dimensions beaucoup plus petites de son corps et par la grandeur sensiblement plus considérable des spicules. (Chez les *H. mansioni* les spicules ont jusqu'à 0·68 mm. et 0·315 mm. de longueur et le spicule droit n'est que deux fois plus court que le spicule gauche, tandis que chez les *H. seurati* le spicule droit n'a pas tout à fait un quart de la longueur du spicule gauche.) Par la longueur de son spicule gauche le *Habronema seurati* rappelle quelque peu *H. unilateralis* Mol. duquel il se distingue cependant sous tous les autres rapports, de sorte qu'il ne sera guère possible de confondre ces deux espèces.

C. Fam. *HETERAKIDAE* Railliet et Henry 1914.

III. Gen. *Ascaridia* Duj. 1845.

3. *Ascaridia hermaphrodita* (Fröhl. 1789).

Littérature. Fröhlich (1789). *Naturforscher*, xxiii. *Ascaris hermaphrodita.*——Zeder (1800). *Erster Nachtrag z. Naturg. d. Eingeweidewürmer.*——Dujardin (1845). *Hist. natur. d. Helminthes*, Paris. *Ascaris truncata.*——Diesing (1851). *Systema Helminth.* ii. 183. *Ascaris truncata.*——Schneider (1866). *Monographie der Nematoden*, Berlin, p. 71, Taf. iii. Fig. 13. *Heterakis truncata.*——Stossich (1888). Il Genere *Heterakis* Duj., Trieste, p. 5. *Heterakis truncata.*——Railliet et Henry (1914). *IX Congrès internat. Zoologie*, Monaco, p. 677. *Ascaridia hermaphrodita* (Fröhl. 1789).

Pl. XVIII, fig. 8.

Dans la collection du prof. N. A. Cholodkowski se trouve la préparation N. 135 avec l'exemplaire unique du mâle de cette espèce (parasite du perroquet *Psittacus* sp.). La localisation du parasite n'est pas indiquée (probablement l'intestin).

Mâle. Longueur totale du corps, 19·0 mm. ; largeur maximale de la partie antérieure du corps, un peu en arrière du bout de l'œsophage est 0·75 mm. A la hauteur où l'œsophage aboutit dans l'intestin, la largeur du corps atteint 0·6 mm., dans la région de l'anus 0·34 mm.

Les lèvres très fortes, au nombre de trois, sont de grandeur presque égale. Le pore excréteur est disposé à une distance de 0·255 mm. du bout céphalique. L'œsophage a l'aspect d'un tuyau musclé s'élargissant graduellement en arrière, mais sans former de bulbe. La longueur de l'œsophage est de 1·36 mm. et la largeur maximale dans la partie postérieure est de 0·255 mm. La largeur de l'intestin est égale à la largeur de l'œsophage.

L'anus se trouve à une distance de 0·323 mm. de l'extrémité caudale.

L'extrémité caudale se rétrécit peu à peu et se termine par un petit effilé à bout arrondi. La bourse caudale est assez peu développée. Treize paires de papilles : six paires préanales et sept paires postanales. Ces papilles sont disposées d'une façon irrégulière et leur grandeur est inégale. Elles sont distribuées de la façon suivante : (*a*) Postanales : 1ère et 2e papilles, de grandeur presque égale, sont disposées l'une au-dessus de l'autre et plus près de la ligne médiane du corps ; 3e papille, la plus petite de toutes, est disposée un peu plus latéralement que les deux premières ; 4e et 5e papilles, de grandeur presque égale, se trouvent au-dessus de la troisième petite ; 6e et 7e papilles, les plus fortes de toutes, ont l'aspect de formations irrégulièrement ovales, en vésicules, elles sont situées en biais l'une à l'autre et la 7e se trouve plus latéralement que la 6e ; ces deux papilles sont tout près de l'anus, de sorte qu'elles peuvent être appelées papilles paranales. (*b*) Préanales : 1ère, 2e et 3e papilles préanales se trouvent presque sur la même ligne horizontale ; la 2e et la 3e étant situées plus latéralement et si près l'une de l'autre qu'à l'examen superficiel elles peuvent passer pour une seule papille double.

Les papilles 4e et 6e sont l'une au-dessus de l'autre, la 5e entre les deux précédentes mais plus latéralement.

La ventouse est pourvue d'un anneau en chitine ; le diamètre de la ventouse atteint 0·22 mm.

Les deux spicules de grandeur égale, longs de 1·87 mm. Leur forme est très caractéristique : le spicule commence par un manche, dont la surface est rayée transversalement, les rayures formant sur les côtés un bord dentelé (environ 25–30 dentelets). Plus tard les bords latéraux deviennent lisses, mais la partie moyenne du spicule s'élargit, formant un appendice en aile unilatéral. Dans la région du plus fort élargisse-

ment du spicule, en face de l'appendice en aile déjà designé, il se trouve une rangée de 10–12 petites dents saillantes.

Dans sa partie postérieure le spicule perd son appendice en aile et se termine par un bout arrondi.

D. Fam. *FILARIIDAE* Claus 1885.

IV. Gen. *Filaria* Müller 1787 (sens. lato).

4. *Filaria coronata* Rud. 1809.

Littérature. Gmelin. *Syst. natur.* (1790), p. 3033, No. 33. *Ascaris coraciae.*——Zeder. *Naturgesch.* (1803), p. 119. *Filaria coraciae.*——Rudolphi (1809). *Entoz.* II. i. 65. *Filaria coronata.*——(1819). Synopsis, p. 6, No. 15. *Filaria coronata.*——Dujardin (1845). *Hist. nat. d. Helminth.* Paris, p. 55, No. 10. *Filaria coronata* Rud. 1809.——Diesing (1851). *Syst. Helminth.* II. 275. *Filaria coronata* Rud.——Molin (1858). *Monograph. d. Filar.* pp. 408–409. *Filaria coronata* Rud.——Linstow (1886). *Voyage de Fedtschenko au Turkestan*, Moscou, p. 11.——(1901). Beobacht. an Helminth. etc. *Archiv f. mikrosk. Anat.* LVIII. 189–190, Fig. 14.

Pl. XIX, figs. 14, 15.

Hôte : *Caracias garrula.*

Localisation : sous la peau du cou.

Distribution géographique : Europe.

Dans la collection du prof. N. A. Cholodkowski se trouve un exemplaire de la femelle de cette espèce long de 32 mm. sur une épaisseur maximum de 0·7 mm. Bouche limitée par six lèvres festonnées, faiblement accentuées. Deux papilles latérales et huit papilles sub-médianes à la tête. L'œsophage est droit, cylindrique, long de 1·275 mm. sur une épaisseur de 0·12 mm. L'anus est à une distance de 0·11 mm. de l'extrémité postérieure du corps. L'extrémité caudale est arrondie. L'orifice génital est fortement proéminent en phyme génital, disposé dans la partie antérieure du corps, à une distance de 0·61 mm. de l'extrémité céphalique (d'après Dujardin à une distance de 0·5 mm.); il atteint 0·17 mm. de largeur, et il est légèrement étréci près de l'orifice génital. L'anneau nerveux est disposé à une distance de 0·221 mm. de l'extrémité céphalique. L'épaisseur du corps dans la région terminale de l'œsophage atteint 0·46 mm., au niveau de l'orifice génital 0·34 mm. et 0·17 mm. au niveau de l'anus.

Les anses postérieures des glandes génitales se trouvent à une distance de 0·68 mm. de l'extrémité caudale. Les œufs sont ovales aplatis, légèrement courbés à leur grand axe. La longueur des œufs est de 0·058 mm. sur une épaisseur de 0·037 mm.

A mon grand regret je n'ai pas eu à ma disposition de mâle de cette espèce, l'étude duquel aurait aidé à éclaircir la parenté de *Filaria coronata* avec les autres représentants du genre *Filaria*.

Pour plus de clarté j'ai cru utile de donner une brève description des mâles de cette espèce, d'après les données de Linstow.

Le *mâle*, selon les données de Linstow (1901), atteint 16 mm. de longueur sur une épaisseur de 0·51 mm. La queue forme $\frac{1}{225}$e partie de la longueur du corps. Directement en avant du cloaque, de même qu'à l'extrémité caudale, se trouve une papille impaire. Les spicules sont de grandeur presque égale: l'un a 0·19 mm. et l'autre 0·22 mm. de longueur. Les extrémités libres des spicules sont renflées en forme de matras. La longueur de l'œsophage chez le mâle est $\frac{1}{225}$e de la longueur totale du corps.

La littérature ne possède qu'une figure de l'extrémité caudale du mâle de ce parasite (Linstow 1901). J'ai cru donc utile de donner ici les figures des parties céphalique et caudale de la femelle.

V. Gen. *Aprocta* Linstow 1883.

Jusqu'au dernier temps on attribuait au genre *Aprocta* Linstow les espèces suivantes:

1. *A. cylindrica* Linst. 1883 de *Petroeca cyanea*.
2. *A. narium* Linst. 1901 de *Buteo* sp.
3. *A. orbitalis* Linst. 1901 de *Falco fuscoater*.
4. *A. turgida* Stoss. 1902 de *Larus argentatus*.
5. *A. ophthalmophaga* Stoss. 1902 de *Falco* sp.
6. *A.* (*Lissonema*) *rotundata* (Linst. 1903) de *Centropus sinensis*.
7. *A. crassa* Raill. et Henry 1910 de *Otis tarda*.
8. *A. matronensis* Raill. et Henry 1910 de *Corvus cornix*.

A part de ces huit espèces je trouve nécessaire d'attribuer à ce même genre encore un parasite—*Spiroptera aerophila* Linstow 1906 de la trachée du flamingue—*Phoenicopterus roseus*. Tous les caractères de ce parasite correspondent parfaitement à ceux des autres espèces du genre *Aprocta*; comme une apparente exception on peut signaler seulement la présence chez les *Spiroptera aerophila* de deux papilles postanales, tandis que dans le diagnostic du genre *Aprocta* donné par Linstow (en 1883 et 1905) et par Railliet et Henry (1910) il est dit: "le mâle n'a pas de papilles caudales." Pourtant dans la description de l'espèce typique de ce genre—*Aprocta cylindrica* Linst. 1883—nous trouvons une indication sur la présence d'une

paire de papilles postanales. En vue de quoi j'ai fait une petite correction: au lieu d'affirmer que "chez le mâle il n'y a pas de papilles caudales," je dis "il n'y a pas de papilles préanales; les papilles postanales sont absentes ou bien se trouvent en petit nombre (1–2 paires). De telle sorte je compte le parasite *Spiroptera aerophila* Linst. 1906 comme un neuvième représentant du genre *Aprocta* Linst."

9. *A. aerophila* Linst. 1906 du *Phoenicopterus roseus*.

5. *Aprocta turgida* Stoss. 1902.

Littérature. Stossich (1902). Nematodi Collezione Parona. *Atti d. Soc. Ligustica di Sc. natur.* Genova, xiii. 72–73 (sans figures).——Railliet et Henry (1910). Deux espèces nouvelles du genre *Aprocta* Linst. *Bull. de la Soc. de Pathol. Exotique*, iii. 152.——K. I. Skrjabin (1916). Matériaux pour servir à une monographie des nématodes d'oiseaux, i. *Aprocta. L'annuaire du Mus. Zool. de l'Acad. d. Sciences*, Pétrograd, xxi. 120–122, Figs. 1–2.

Pl. XIX, figs. 16—19.

L'hôte: *Larus argentatus*.

Localisation: région nasale.

Description de l'espèce:

Dans son travail de 1902 Stossich ne consacre que sept lignes au nématode *Aprocta turgida* parasite dans la région nasale de la mouette *Larus argentatus*; la femelle de ce parasite il décrit de la façon suivante: elle est longue de 20–27 mm., large de 1·0 mm.; un corps gros, cylindrique, lisse; les extrémités céphalique et caudale sont arrondies; la bouche dépourvue de lèvres, ni papilles ou capsule buccale; presque tout le corps du parasite est bombé d'une quantité d'œufs ovales, à coque épaisse, lisse, renfermant des embryons formés.

Malheureusement, Stossich ne cite pas des données numériques, qui pourraient être utiles pour comparer ce parasite aux espèces apparentées; il se borne à la caractéristique ci-dessus; le dessin de l'espèce manque également.

Dans la collection helminthologique du Musée Zoologique de l'Académie des Sciences de Pétrograd j'ai réussi à découvrir le parasite *Aprocta turgida* Stoss. 1902 dans la région nasale de *Larus* sp. (M. le Dr Panow leg. 11. v. 1911, tube N 525), et dans ce tube se trouvaient non seulement des femelles, mais aussi des mâles, qui n'ont jamais été décrits jusqu'à présent. La description ci-dessous est basée sur l'examen de ces exemplaires de musée.

Le corps du parasite est de couleur blanche, aux extrémités antérieure et postérieure arrondies; la cuticule est finement rayée dans le sens

longitudinal, les rayures transversales ne sont pas apparentes. Sur l'extrémité céphalique il n'y a ni papilles, ni organes analogues aux lèvres.

Le *mâle* atteint 20–24 mm. de longueur et sa largeur maximale est de 1·02 mm. dans la partie antérieure du corps ; la largeur du corps au niveau du cloaque atteint 0·255 mm.

L'extrémité caudale est tournée en spirale ; on n'a pu découvrir aucune papille dans la région caudale du mâle. Deux spicules égaux atteignant 0·272 mm. de longueur, ont une forme recourbée en sabre ; leur bout antérieur est un peu élargi et a une surface jarreuse pour l'attache des muscles rétracteurs. L'examen à un fort grossissement montre dans chaque spicule une région centrale, limitée par ses lobules de côté retournés ; un lobule dépasse l'autre de sorte qu'il se forme non un conduit, mais une cavité fermée de côté. Le bout postérieur des spicules est obtusément arrondi. La distance entre l'orifice du cloaque et l'extrémité caudale est de 0·765 mm. Les tubes génitaux forment une spirale extrêmement compliquée autour de l'intestin dans la partie postérieure du mâle.

La *femelle* des exemplaires étudiés par moi atteignait 38 mm. de longueur et 1·2 mm. de largeur maximale dans la partie moyenne du corps. (Les exemplaires de Stossich étaient un peu plus courts—jusqu'à 27 mm.) Au niveau de la vulve la largeur du corps était 0·5 mm., dans la région de l'extrémité caudale, 0·34 mm.

La vulve était située à 1·03 mm. de l'extrémité céphalique. La longueur des œufs atteignait 0·058 mm. et leur largeur 0·034 mm.

6. *Aprocta orbitalis* Linst. 1901.

Littérature. Linstow (1901). Beobachtung. an Helminthen d. Senckenberg. naturhistor. Museums, etc. *Arch. f. mikroskop. Anat.* Bonn, LVIII. 188–189, Taf. VIII. Figs. 10–11.——Railliet et Henry (1910). Deux espèces nouvelles du genre *Aprocta* Linstow. *Bull. Soc. Pathol. Exotique*, III. 155.——K. I. Skrjabin (1916). Matériaux pour servir à une monographie des nématodes d'oiseaux, I. *Aprocta. Annuaire du Mus. Zoolog. de l'Académie d. Sciences*, Pétrograd, XXI. 119–128.

Jusqu'à présent ce parasite n'a été trouvé qu'une seule fois dans la cavité ophtalmique de *Falco fuscoater*, il a été décrit par Linstow en 1901.

Dans la collection du prof. Cholodkowski s'est trouvé un exemplaire du mâle de cette espèce d'un nouvel hôte, *Aquila naevia*, trouvé en Russie (tube N 250). Je me permettrai de donner ici une brève

caractéristique de ce parasite, d'autant plus que quelques détails de son organisation méritent notre attention.

Le *mâle* que j'ai étudié atteignait 25 mm. de longueur sur une épaisseur maximum de 0·95 mm. L'épaisseur du corps dans la région terminale de l'œsophage est de 0·52 mm., 0·3 mm. dans la région du cloaque. La cuticule a de délicates striures transversales. Les striures sont distancées de 0·001 mm. L'œsophage a 0·884 mm. de longueur. L'anneau nerveux se trouve à 0·187 mm. de l'extrémité céphalique. L'extrémité céphalique est arrondie sans lèvres ni papilles. L'extrémité caudale est aussi arrondie. L'anus est disposé à 0·32 mm. de l'extrémité caudale. Deux spicules de grandeur inégale, 0·49 mm. et 0·40 mm. (d'après Linstow = 0·47 et 0·40 mm.). L'orifice du cloaque est légèrement proéminent, ce qui d'ailleurs trouve aussi lieu chez d'autres *Aprocta* (je l'ai observé chez *Aprocta turgida* Stoss.).

Une particularité caractéristique de cette espèce est la présence sur la partie ventrale du corps, directement en avant du cloaque, de petites écailles chitineuses disposées en rangs transversaux. Cette particularité qui n'a été notée par aucun des auteurs qui ont étudié les représentants du genre *Aprocta* est extrêmement intéressante, car elle pourra probablement servir de caractère important pour distinguer *Aprocta orbitalis* des autres espèces de ce genre. L'extrémité caudale est tordue deux fois et a tout à fait le même aspect que chez *Aprocta turgida* (voir Pl. XIX, fig. 19). Les tubes génitaux se tortillent en spirales irrégulières dans la partie postérieure du mâle.

Sub-fam. Diplotriaeninae nov. sub-fam.

VI. Gen. *Diplotriaena* Railliet et Henry 1909.

7. *Diplotriaena bargusinica* nov. spec.

Pl. XVIII, figs. 9, 10.

Hôte : *Turdus* sp. ; probablement dans la cavité abdominale.

Distribution géographique : La Sibérie, la région au delà du Baïkal (expédition du fleuve Bargusa).

Collection du professeur N. A. Cholodkowski.

Le corps est cylindrique, blanc, légèrement atténué des deux bouts. Le *mâle* atteint ca. 45 mm. de longueur et une largeur maximale de 0·68 mm. Dans la région où se trouve l'anneau nerveux le corps atteint une largeur de 0·3 mm., au niveau du trident 0·238 mm.

et à la hauteur du cloaque 0·374 mm. Deux papilles latérales et quatre papilles submédianes faiblement perceptibles se trouvent sur la tête. Le trident chitineux est disposé de telle manière que son anse joint presque complètement l'ouverture buccale. La longueur du trident = 0·102–0·119 mm. ; les parties postérieures des rameaux du trident sont légèrement renflées et ont des contours toruleux.

L'œsophage est composé de deux parties : l'une plus petite, rétrécie, atteignant 0·289 mm. de longueur et une largeur de 0·085 mm. et l'autre, plus large allongée, atteignant 4·0 mm. de longueur, ayant 0·3 mm. de largeur à la base. A la limite qui sépare ces deux parties l'œsophage est entouré d'un anneau nerveux, disposé à une distance de 0·255 mm. du bout céphalique. La largeur de l'intestin varie entre 0·255–0·374 mm. dans ses différentes parties. La structure de son œsophage rapproche cette espèce à plusieurs autres espèces des *Diplotriaena* (par exemple *D. tricuspis* Fedtschenko), d'un autre côté elle rapproche le genre des *Diplotriaena* du genre *Contortospiculum* Skrjabin 1915.

Le bout postérieur du mâle est obtusément arrondi. Le cloaque est disposé à une distance de 0·238 mm. du bout postérieur. Les spicules au nombre de deux, sont de forme et de grandeur inégales. Le grand spicule est droit, il atteint 0·7 mm. de longueur, ayant une largeur de base de 0·051 mm. En l'examinant attentivement on peut apercevoir que ce spicule est composé d'une mince lance raidement enroulée en longueur à la suite de quoi elle a pris une forme cylindrique spiralée avec un canal central étroit. L'autre spicule, plus petit, atteint 0·544 mm. de longueur, ayant une largeur de base de 0·034 mm. ; il a la forme spiralée caractéristique pour le genre des *Diplotriaena*, l'un de ses bords est accompagné d'une mince membrane transparente.

Le grand spicule de cette espèce rappelle par sa structure les représentants du genre *Serratospiculum* Skrjabin 1915 et *Contortospiculum* Skrjabin, chez qui le plus grand spicule a aussi la torsion longitudinale de sa partie foliacée ; cependant chez les représentants de ces deux derniers genres la torsion ne va jamais jusqu'à la formation d'un corps cylindrique, comme cela a lieu chez les *Diplotriaena bargusinica* n. sp.

Je n'ai pu découvrir chez cette espèce aucune trace de papilles caudales.

La *femelle* atteint 85–92 mm. de longueur, ayant 1·0 mm. de largeur. La largeur du corps dans la région du trident chitineux atteint 0·34 mm.,

dans la région de la vulve 0·646 mm. Il est intéressant de noter que le trident chitineux de la femelle a les mêmes dimensions que le trident du mâle, quoique la femelle soit beaucoup plus grosse que le mâle. L'ouverture génitale extérieure se trouve à une distance de 0·34 mm. du bout céphalique. Les œufs sont ovales, ils ont une longueur de 0·068 mm. et une largeur de 0·04 mm.

VII. Gen. *Contortospiculum* Skrjabin 1915.

Dans mon récent ouvrage[1] j'ai établi pour toute une série de Nématodes figurants dans la littérature comme des représentants du genre *Filaria* Müll. 1787, un nouveau genre, *Serratospiculum* n. g., ayant pris pour type *S. turkestanicum* Skrjabin 1915 du sac aérifère pectoral de *Falco tinnunculus*.

Dans la collection helminthologique du Cabinet Zoologique de l'Académie Impériale Militaire de Médecine, parmi les préparations qui m'ont été gracieusement offertes à étudier par le prof. N. A. Cholodkowski s'est trouvé un parasite de la cavité ventrale de l'outarde *Otis tarda*. En l'examinant de plus près j'ai constaté qu'il est tout à fait semblable à l'espèce *Filaria horrida* Dies. 1851, qui est un parasite de la cavité ventrale de l'autriche américain, le nandu (*Rhea americana*). A la première vue l'identité des parasites européen et américain n'aurai semblé que peu probable. Il m'était pourtant possible de confirmer l'exactitude de ma supposition par l'étude personnelle de *Filaria horrida*. Ce parasite m'a été aimablement envoyé à déterminer par mon collègue K. D. Michailoff qui l'a trouvé dans la cavité ventrale et dans l'intestin du *Rhea americana* à l'Askania Nova (Zoopare de Falz-Fein), gouvernement de la Tauride.

L'étude de la littérature relative à cette question m'a montré l'existence d'une parenté proche entre l'espèce *Filaria horrida* Dies. et *Filaria labiata* Creplin 1825, parasite de la cavité ventrale de la cigogne blanche et noire (*Ciconia alba* et *Ciconia nigra*). Outre cela, l'étude comparée de ces espèces m'a permis de trouver de caractères communs, qui m'ont servi de base pour exclure ces deux parasites du genre *Filaria* et de les placer dans un genre nouveau indépendant que je dénomme, en me basant sur le caractère de son grand spicule, *Contortospiculum* nov. gen. Je considère comme type de ce genre l'espèce *Filaria horrida*

[1] K. I. Skrjabin (1915). Nématodes des oiseaux du Turkestan russe. *Annuaire du Mus. Zool. de l'Acad. de Sciences de Pétrograd*, xx.

Dies. 1851, qui à présent devra se dénommer *Contortospiculum horrida* (Dies. 1851); *Contortospiculum labiata* (Creplin 1825) sera une seconde espèce de ce genre; ce dernier parasite a été décrit par Gmelin en 1791 sous le nom de *Filaria ciconiae*, à la suite de quoi il devra selon les règles de la priorité se dénommer *Contortospiculum ciconiae* (Gmel. 1791).

Le présent travail a pour but de donner les caractères de ce nouveau genre en rapport avec la description des espèces qui lui appartiennent.

En décrivant le parasite *Contortospiculum horrida* (Dies. 1851), je me base sur mes propres recherches, qui en parties ne coïncident pas avec celles de Linstow, faites en 1897 (il est question de la structure des ornementations céphaliques et du caractère des spicules).

Pour conclure j'ai tenté d'établir le lien phylogénétique entre mon nouveau genre et les autres genres des filaires.

Genre *Contortospiculum* Skrjabin 1915.

Diagnose. Grands Nématodes de la famille *Filariidae* Claus 1885 caractérisés par une ornementation céphalique spéciale: l'orifice buccal est limité des côtés par deux lèvres saillantes très fortes; à limite postérieure de chaque lèvre est contiguë une formation en épaulette, concave dans sa partie médiane et formant du côté latéral trois lobes: dorsal, mitoyen et ventral. Deux papilles latérales et huit papilles submédianes; quatre de ces dernières sont disposées sur les formations en épaulette (deux papilles sur chacune). L'œsophage consiste de deux parties: la partie antérieure mince et courte, et la partie postérieure longue et épaissie. La queue du mâle avec une bourse assez large porte cinq à six paires de papilles préanales pédonculées et quelques paires de papilles postanales. Deux spicules de forme et de dimension inégales; l'extrémité antérieure des deux spicules est renflée et clavelée; la moitié postérieure du grand spicule a un élargissement aliforme, les ailes sont enroulées dans leur direction ventrale, et forment un tube plus ou moins fermé; les bords des ailes du grand spicule sont crénelés, les ailes mêmes sont transversalement striées. Le petit spicule est courbé, sans crénelures. L'orifice génital de la femelle se trouve près de l'extrémité céphalique. Dans les œufs l'embryon est enroulé en cercle. Parasites des cavités séreuses, de tissus sous-cutanées et de la voie digestive des oiseaux. Espèce typique: *Contortospiculum horrida* (Diesing 1851) du *Rhea americana*, *Struthio crux* et *Otis tarda*. Seconde espèce de ce genre: *Contortospiculum labiata* (Creplin 1825) des cigognes: *Ciconia alba* et *Ciconia nigra*.

Au cours de mon exposé je donnerai séparément la description de chacun de ces parasites, en l'accompagnant d'une énumération, aussi complète que possible, de la littérature.

8. *Contortospiculum horrida* (Dies. 1851).

Littérature. Diesing (1851). *Systema Helminth.* II. 278; (1857) *Denkschr. Wien. Akad.* XIII. 19, Taf. III. Figs. 1–5, Taf. IV. Figs. 1–25.——Molin (1858). *Wien. Sitzgbericht.* XXVIII. 416.——Schneider (1866). *Monograph. d. Nematoden*, p. 89, Fig. dans texte.——Linstow (1880). Helmintholog. Untersuch. *Arch. f. Naturgesch.* XLVI. 46 (*Filaria horrida* de la hanche *Struthio crux*).——Leidy (1884). *Proc. Acad. Nat. Sci. Philadelphia*, p. 47.——Carlos Berg (1896). Una filaria horrida Dies. dentro de un Huero. *Ann. Museo Nacional de Buenos Aires*, v. 139–140.——Linstow (1897). Zur Systematik der Nematoden. *Arch. f. mikrosk. Anat.* XLIX. 613, Figs. 12–18.——Stossich (1897). *Filarie e Spiroptere*, Trieste, pp. 48–49, No. 71.

Pl. XVIII, fig. 11, XIX, figs. 12, 13, Fig. 1 (texte).

Hôte: *Rhea americana* [Diesing, Schneider, Linstow, Leidy], *Struthio crux* [Linstow], *Otis tarda* [Skrjabin].

Localisation: cavité ventrale, cavité pectorale, gésier, intestin, muscle de la hanche (Linstow), sous la peau de la hanche, dans l'œuf (Berg).

Distribution géographique: Brésil; en Russie le Zooparc Askania Nova, gouvernement de la Tauride.

Description du parasite: corps long, cylindrique, blanc, très atténué vers l'extrémité caudale, moins vers l'extrémité céphalique. Les deux extrémités sont arrondies. La cuticule est transversalement striée; la striure longitudinale est imperceptible. L'extrémité céphalique est caractérisée par une structure tout à fait spéciale (Fig. 1, schema). Avant tout, l'attention est attirée par deux grosses lèvres saillantes, disposées à gauche et à droite de l'orifice buccal; au côté postérieur de chaque lèvre sont contiguës des formations en épaulette, très originales, disposées latéralement; le bord médian de ces épaulettes est concave et le bord latéral festonné, formant trois lobes, dorsal, mitoyen et ventral. Ces formations ont l'air d'un haut-relief sur les côtés latéraux de l'extrémité céphalique du parasite. L'originalité du dessin est encore augmentée par les papilles, au nombre de dix, disposées de la manière suivante: deux papilles latérales contiguës au bord extérieur des lobes mitoyens des formations en épaulette; huit papilles submédianes, dont quatre, les plus grosses, sont hors des épaulettes, dans les échancrures entre leurs lobes; les autres quatre papilles submédianes sont sur la surface

même des épaulettes sur les lobes dorsaux et ventraux. Je n'ai pas pu observer sur mes préparations les épines du lobe mitoyen dont parle Linstow (1897, p. 613). Linstow, au contraire, n'a pas aperçu les deux papilles latérales, qui, comme je l'ai dit plus haut, sont contiguës à ces lobes mitoyens sur leur bord extérieur. J'ai observé à la surface antérieure de ces lobes deux orifices glandulaires de chaque côté, tandis que Linstow ne décrit qu'un seul orifice.

A l'extérieur des lèvres, Linstow a observé un corps conique, "ein kleiner Kegel," qui est représenté par lui fig. 14, comme une papille pointue; dans mes préparations cette formation a l'air d'un col cadrant la moitié postérieure des lèvres du parasite.

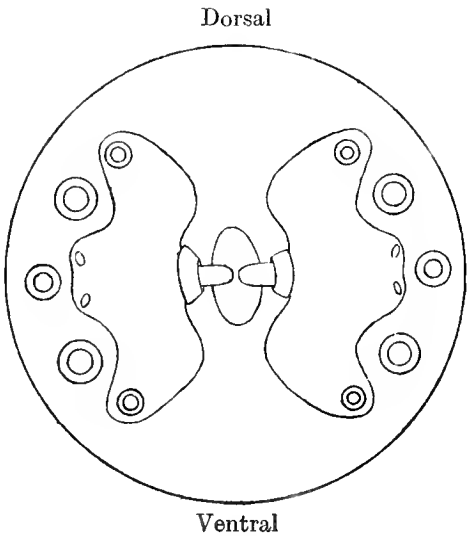


Fig. 1.

La disposition des papilles céphaliques et des épaulettes chez *Contortospiculum horrida* (Dies. 1851). (Schema.)

L'œsophage a une forme spéciale; il est composé de deux parties; la partie antérieure, courbé et étroite (chez le mâle 0·51 mm. de longueur sur 0·136 mm. d'épaisseur), et de la partie postérieure longue et large (chez le mâle 17·0 mm. de longueur sur une épaisseur de 0·68–0·986 mm.). Selon les observations de Linstow, les lumières des deux parties de l'œsophage sont tout à fait égales, il n'y a que l'épaisseur des parois qui soit différente. Dans l'épaisseur des parois de la partie antérieure de l'œsophage, Linstow a observé des conduits qui s'ouvrent à la surface du lobe postérieur des formations en épaulette.

Le *mâle* parasite du nandu, que j'ai examiné, atteignait 176 mm. de longueur sur une épaisseur maximum de 1·13 mm. La longueur du mâle

parasite de l'outarde atteignait 225 mm. Schneider a étudié un mâle long de 130 mm., Diesing de 325 mm. sur 2·26 mm. d'épaisseur et Linstow enfin a vu un mâle de 205 mm. de longueur sur 1·2 mm. d'épaisseur.

La bourse caudale de mes exemplaires avait 0·425 mm. de longueur et 0·27 mm. d'épaisseur. J'ai trouvé cinq paires de papilles préanales (et non pas six paires comme le supposait Linstow), elles étaient toutes pédonculées. Ensuite j'ai observé six petites papilles sessiles (c'est à dire sans tige), dont parle Schneider dans sa monographie (ouvrage de l'an 1866, p. 89). Ces papilles sont disposées de telle manière que la paire mitoyenne est plus rapprochée de la ligne médiane du corps, que la paire antérieure et la paire postérieure. En général le nombre et la disposition des papilles sur mes préparations correspondent bien aux données de Schneider et diffèrent des données de Linstow.

Quatre paires de papilles postanales: trois paires tout près de l'extrémité caudale et la quatrième paire, la plus grosse, sur de grands pédicules, située immédiatement en arrière du cloaque.

Les deux spicules sont de forme et dimension inégaux: le plus grand atteignait chez mon exemplaire 0·9 mm. de longueur (0·95 mm. selon Linstow); la partie antérieure avait la forme d'un pivot, pourvu à son extrémité d'un manche, qui, selon la juste comparaison de Linstow, rappelle une pomme de canne. La partie postérieure du spicule a une partie mitoyenne suite du pivot, et deux ailes latérales enroulées autour de l'axe longitudinal dans la direction ventrale, de sorte que l'aile gauche passe au-dessus de l'aile droite, en la couvrant de sa surface; les ailes sont transversalement striées. Il résulte de cette disposition, que nous avons à l'intérieur du spicule une cavité en forme de rigole formée par la commissure et l'enroulement des deux ailes; l'extrémité postérieure du spicule est légèrement acuminée; les bords latéraux des ailes sont crénelés.

Le petit spicule atteint 0·34 mm. de longueur (0·33 mm. chez Linstow); il a la forme d'une tige légèrement courbée avec un élargissement unilatéral de sa partie postérieure. L'extrémité antérieure est clavelée, la partie postérieure mince et arrondie.

Linstow dans son ouvrage de 1897 ne fait pas d'attention au caractère des spicules, à la suite de quoi la fig. 15 qu'il nous donne, ne correspond pas du tout à la réalité; il n'y a que la forme en pomme de canne à l'extrémité antérieure du petit spicule qui soit correctement saisie. L'orifice anal est à 0·2 mm. de l'extrémité postérieure du corps. L'épaisseur du corps dans la partie postérieure de l'œsophage est de 1·156 mm.; dans la région de l'anus (bourse

incluse) 0·27 mm. L'épaisseur du corps à l'extrémité céphalique (dans les limites de la disposition des épaulettes) est de 0·238 mm. Immédiatement en arrière des formations en épaulette, l'épaisseur du corps augmente rapidement atteignant un maximum de 1·16 mm.

Femelle. La longueur de mon exemplaire (du nandu) atteignait 665 mm. sur une épaisseur de 2·5 mm ; je n'ai pas eu à ma disposition de femelles prises de l'outarde. Selon Linstow la longueur de la femelle atteignait 618 mm. sur une épaisseur de 2·3 mm. Diesing a étudié une femelle longue de 974 mm. sur une épaisseur de 3·39 mm. Schneider enfin décrit une femelle gigantesque longue de presque $1\frac{1}{2}$ mètres, précisément 1350 mm. Les parties buccales de même que l'ornementation céphalique sont tout à fait pareilles à celles du mâle. L'orifice génital est disposé près de l'extrémité céphalique à une distance de 1·0–1·3 mm. de cette dernière. Ovaires et utérus paires. Les œufs larvés à maturité atteignent 0·05 mm. de longueur sur 0·034 mm. d'épaisseur.

Chez la femelle étudiée par Schneider, dont la longueur atteignait 1350 mm. l'orifice génital était disposé à une distance de 2 mm. de l'extrémité céphalique.

Sur le rapport du genre Contortospiculum avec les autres genres de la famille Filariidae.

Comme chez les représentants du genre *Contortospiculum* le caractère le plus important est l'ornementation spéciale de l'extrémité céphalique, il a fallu pour établir leur parenté avec les autres genres des filaires, étudier attentivement la sculpture des parties buccales de ces parasites. Il fallait avant tout trouver chez les autres filaires des parties homologues aux organes des espèces *Contortospiculum*, que j'avais dénommés "formation en épaulette." Telles sont les formations à l'extrémité céphalique des représentants du genre *Serratospiculum* Skrjabin 1915, que tous les auteurs et moi-même à leur nombre prenions pour des lèvres. Dans le diagnostic du genre *Serratospiculum* il est dit : de grands Nématodes de la famille *Filariidae* Claus 1885, caractérisés par la présence à leur tête de six lèvres, disposées par trois de chaque côté latéral. Dorsalement et ventralement ces lèvres sont séparées par une assez grande distance. Près d'elles deux papilles latérales et huit papilles submédianes[1].

[1] Nématodes des oiseaux du Turkestan russe. *Annuaire du Mus. Zool. de l'Acad. de Sciences de Pétrograd*, XX. 1915.

Il est à remarquer que dans la littérature helminthologique, le mot "lèvre" n'est pas un terme spécifique, déterminé en rapport avec une structure déterminée, ou avec une fonction physiologique. Les helminthologues donnent le nom de "lèvres" à toutes les proéminences cuticulaires qui limitent l'orifice buccal. C'est justement dans ce sens que le nom de lèvres a été appliqué à ces premières formations latérales trilobées du genre *Serratospiculum*, disposées latéralement par rapport à l'orifice buccal.

Cependant en examinant attentivement la structure de ces lèvres, surtout en examinant l'extrémité céphalique du *Serratospiculum* vu de la face antérieure, il nous vient involontairement l'idée, que ces "six lèvres disposées par trois de chaque côté latéral" sont homologues aux formations en épaulette du *Contortospiculum*, qui "du côté latéral forment trois lobes: le lobe dorsal, ventral et mitoyen"; en quoi il sera facile de s'assurer, en comparant les dessins illustrant les extrémités céphaliques des représentants de ces deux genres vues de la surface antérieure.

Cette conclusion nous donne le droit de parler d'une parenté entre les genres *Contortospiculum* et *Serratospiculum*, ce qui est encore affirmé par la présence chez eux de deux papilles latérales et de huit papilles submédianes céphaliques. Le caractère des spicules (deux spicules inégaux, le plus grand est aliformement élargi) de même que quelques particularités biologiques (le parasitisme surtout dans les cavités séreuses) témoignent aussi la parenté de ces genres.

Ainsi les organes des espèces *Serratospiculum* que j'ai dénommés de concert avec tous les helminthologues précédents comme "lèvres" sont quelques formations spécifiques, probablement de nature chitineuse dont le rôle physiologique n'est pas encore établi. Je les nommerai "formations en épaulette[1]."

Ayant établi l'homologie des parties buccales des représentants du genre *Serratospiculum* et *Contortospiculum*, il nous vient l'idée que le mystérieux trident chitineux disposé latéralement par rapport au bord antérieur de l'œsophage chez les espèces du genre *Diplotriaena* Railliet et Henry 1909 est aussi homologue aux "formations en épaulette." C'est vrai qu'il y a une assez grande différence entre "les épaulettes" et le trident; mais je crois que cette différence est plutôt

[1] Il sera donc nécessaire de corriger le diagnostic du genre *Serratospiculum* comme il suit: au lieu de "à la tête six lèvres disposées par trois de chaque côté latéral," il faudra mettre "des côtés de l'orifice buccal deux formations en épaulette divisées en trois lobes dorsal, mitoyen et ventral."

quantitatif que qualitatif. Je considère comme preuves de cette homologie que : (1) la disposition du trident latéralement de la ligne médiane du corps correspond complètement à la disposition des épaulettes ; (2) la présence de trois branches chez chaque trident, dont l'une a une disposition dorsale, l'autre mitoyenne et la troisième ventrale, correspond complètement aux trois lobes des épaulettes, le lobe dorsal, mitoyen et ventral ; (3) l'anse du trident n'a pas encore perdu son rapport avec la cuticule extérieure sous laquelle elle ressort quelquefois en forme de papille ; la disposition de la sommité de l'anse tridentaire correspond exactement à la position des épaulettes, c'est à dire des deux côtés de l'orifice buccal.

Il reste à admettre que le trident du genre *Diplotriaena* provient des épaulettes du genre *Serratospiculum* et *Contortospiculum* par voie d'immersion de ces derniers à l'intérieur du corps ; le rapport du trident avec la superficie extérieure n'est pas complètement perdu, car l'anse de ce dernier soulève très souvent, sous forme de papille, la couche cuticulaire.

Je suppose donc que les trois genres des filaires (1) *Diplotriaena* Railliet et Henry 1909, (2) *Serratospiculum* Skrjabin 1915, et (3) *Contortospiculum* Skrjabin 1915, se distinguent de tous les autres représentants de la famille *Filariidae* par la présence de particulières formations chitineuses, trilobées disposées des côtés de l'orifice buccal, ayant une forme différente (trident, épaulette), mais homologues entre elles. La parenté de ces trois genres se manifeste aussi dans le caractère plus ou moins monotypique des spicules (deux spicules inégaux, le plus petit ayant une forme courbée) et dans une ressemblance biologique : le parasitisme dans les cavités séreuses des oiseaux. Tout ceci m'autorise de réunir ces trois genres de filaires *en une sous-famille particulière qui devra se dénommer* Diplotriaeninae *Skrjabin* 1915.

Le diagnostic de Sub-fam. DIPLOTRIAENINAE sera comme il suit :

De grands filaires, du côté de l'orifice buccal desquels sont disposées des formations chitineuses ayant une tendance à un démembrement en trois parties. Ces formations peuvent être disposées aussi bien à la surface de la cuticule qu'à l'intérieur du corps des côtés de l'œsophage. Deux spicules de grandeur inégale, le plus grand est plus ou moins fortement courbé. Parasites des cavités séreuses des oiseaux.

Genre typique Diplotriaena *Railliet et Henry* 1909.

Autres genres : Serratospiculum *Skrjabin et* Contortospiculum *Skrjabin* 1915.

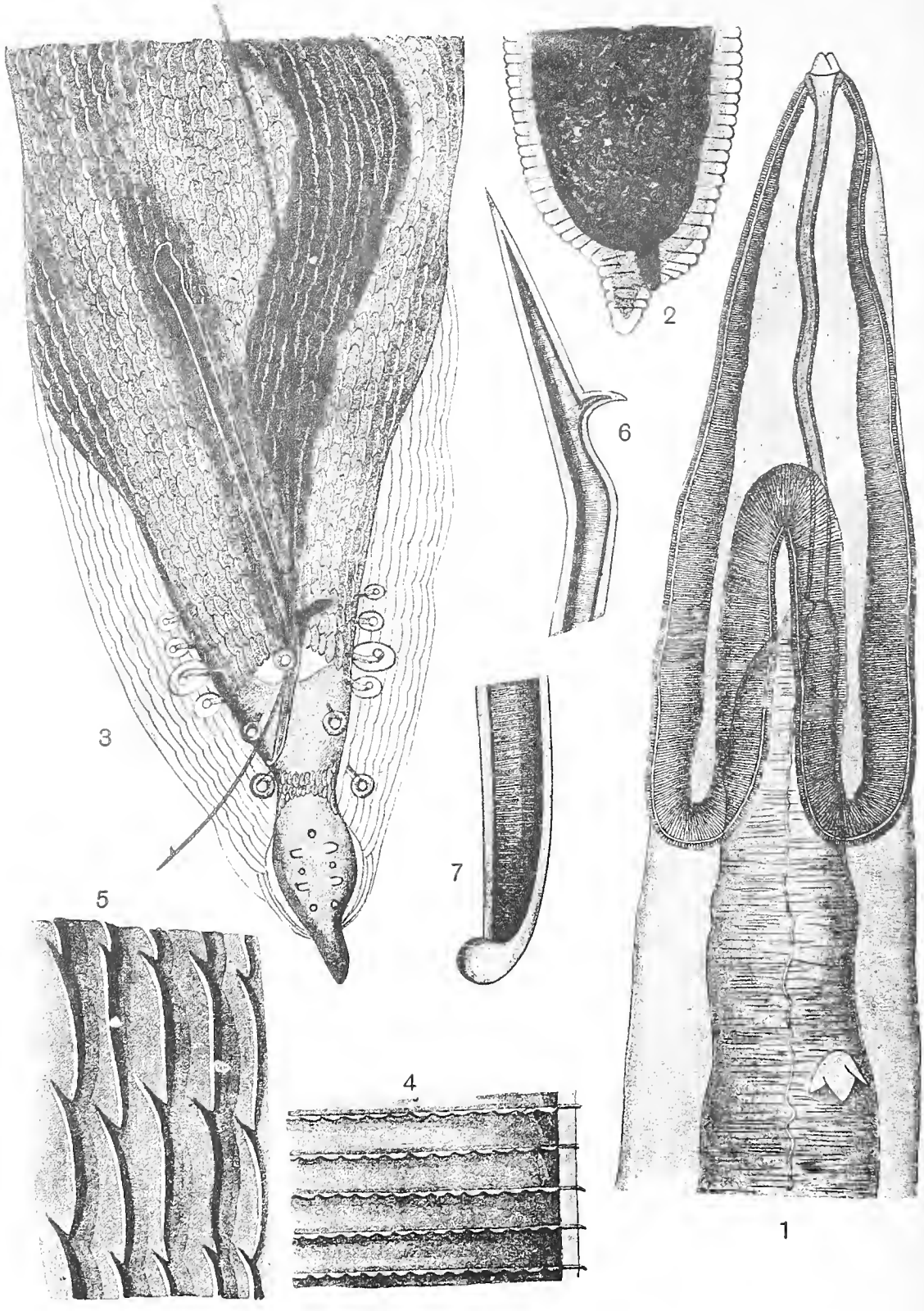
EXPLICATION DES PLANCHES XVIII ET XIX.

PLANCHE XVIII.

Fig. 1. *Acuaria* (*Synhimanthus*) *brevicaudatus* Duj. 1845. L'extrémité céphalique de la femelle.
Fig. 2. La queue de la femelle.
Fig. 3. *Habronema seurati* nov. spec. L'extrémité caudale du mâle.
Fig. 4. La striation transversale du corps; vue du côté.
Fig. 5. La seulpture de la eutieule à la faee ventrale de la queue du mâle.
Fig. 6. L'extrémité postérieure du spicule gauehe.
Fig. 7. L'extrémité postérieure du spicule droite.
Fig. 8. *Ascaridia hermaphrodita* (Fröhl. 1789). La queue du mâle.
Fig. 9. *Diplotriaena bargusinica* nov. spec. L'extrémité eéphalique du mâle.
Fig. 10. La queue du mâle avec deux spieules.
Fig. 11. *Contortospiculum horrida* (Dies. 1851) de *Otis tarda* L. L'extrémité céphalique.
Fig. 12. Le mâle de *Contortospiculum horrida* (Dies. 1851) de *Otis tarda* L Grandeur presque naturelle (Photographie).

PLANCHE XIX.

Fig. 13. La queue du mâle de la mêmc espèce.
Fig. 14. *Filaria coronata* Rud. 1809. La queue de la femelle.
Fig. 15. L'extrémité céphalique de la femelle.
Fig. 16. *Aprocta turgida* Stoss. 1902. Le spieule du mâle.
Fig. 17. Le mâle et la femelle, grandeur naturelle.
Fig. 18. L'extrémité eéphalique de la femelle.
Fig. 19. La queue du mâle.



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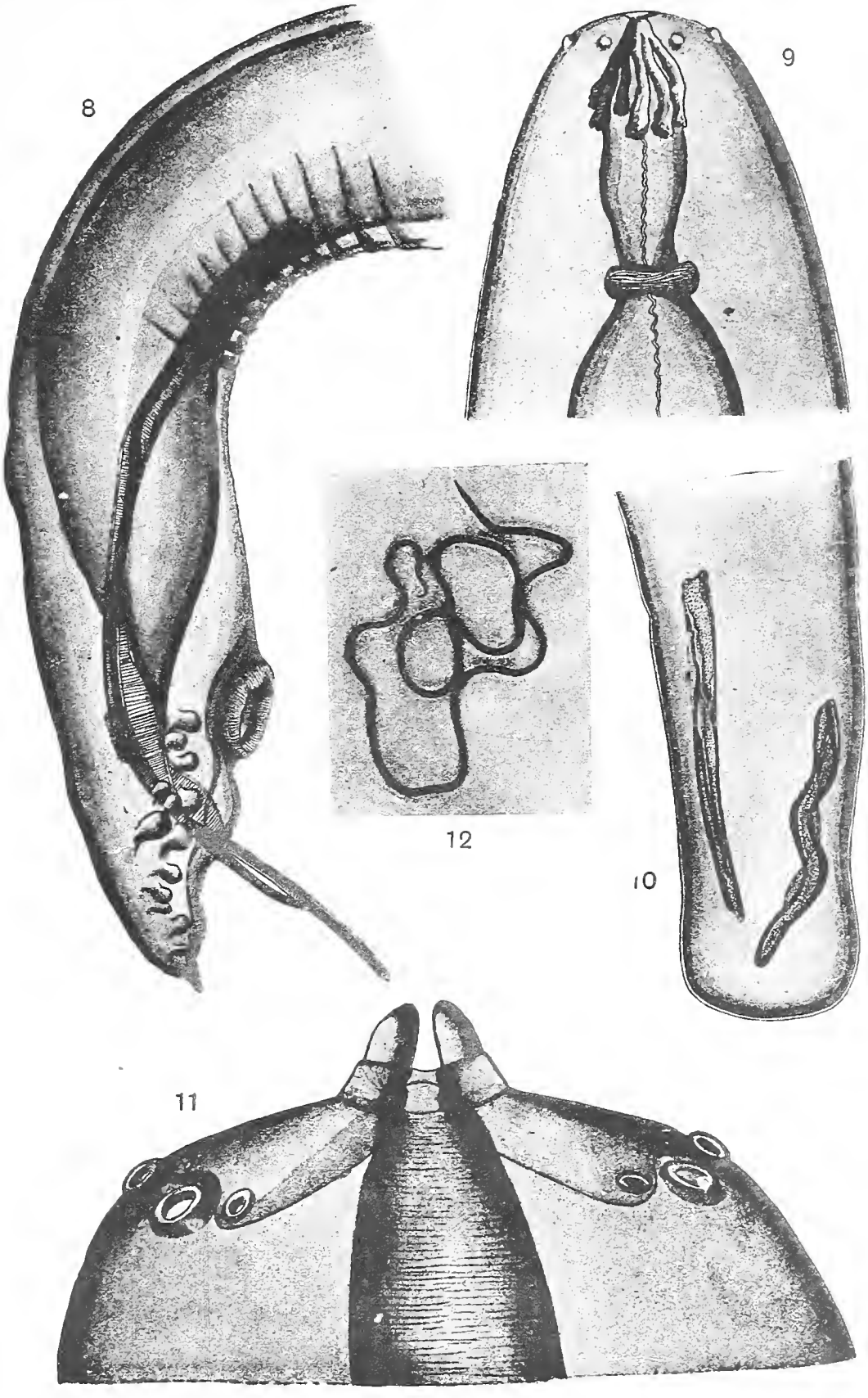
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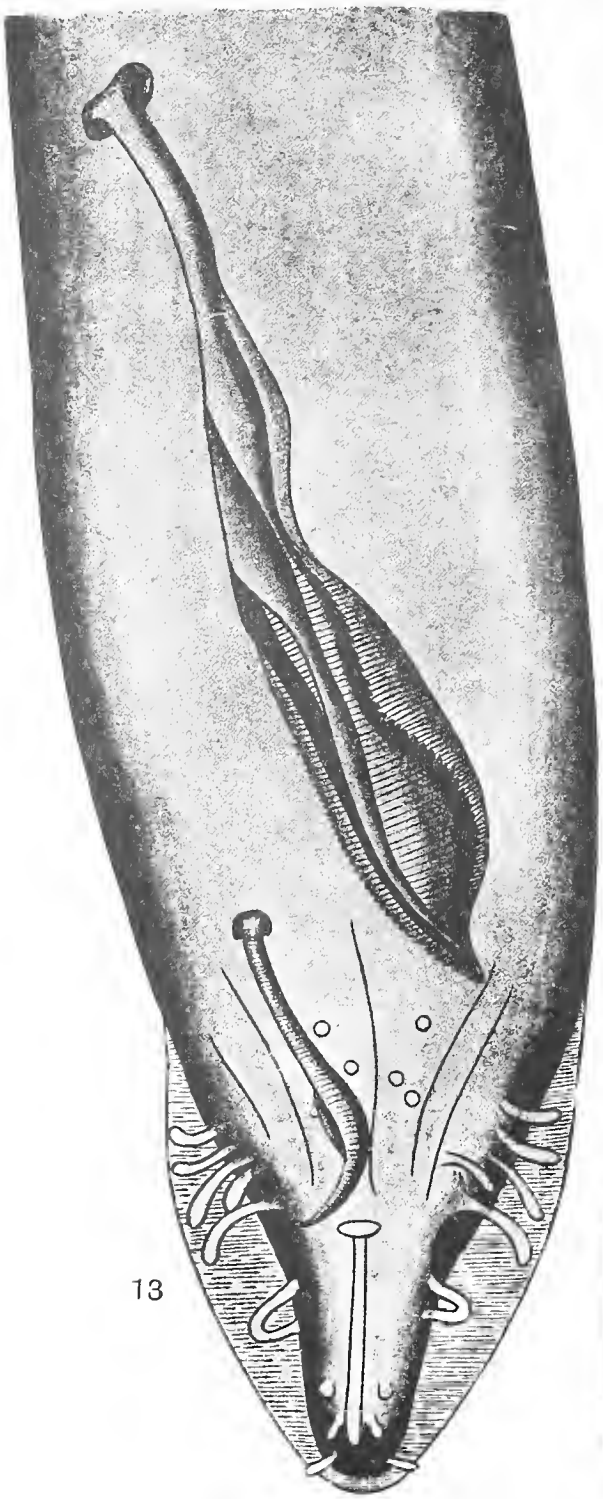

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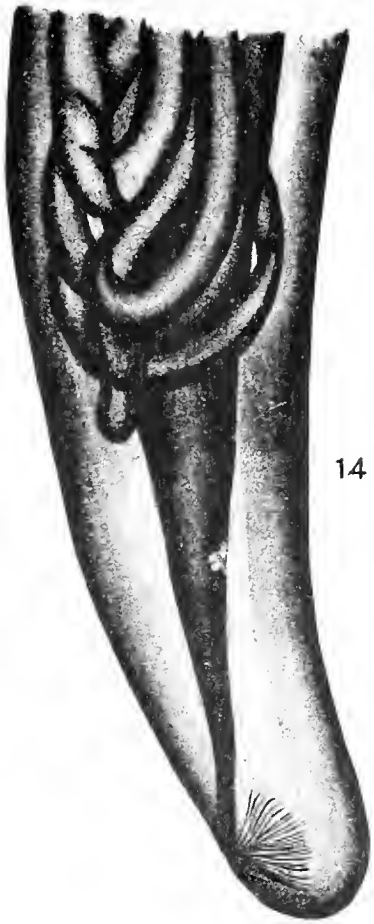

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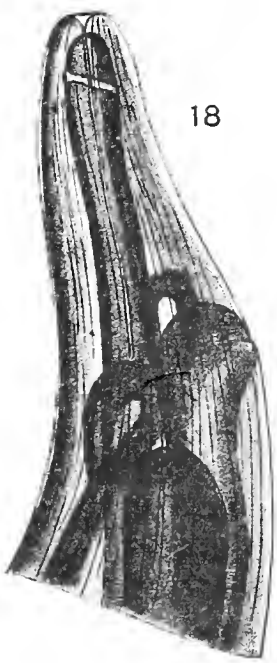

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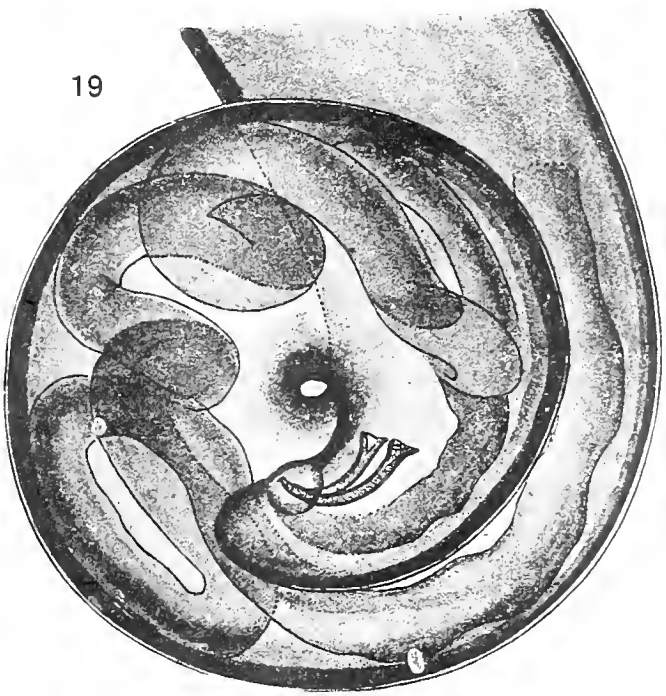

THE RELATION BETWEEN THE HATCHING OF THE EGGS AND THE DEVELOPMENT OF THE LARVAE OF *STEGOMYIA FASCIATA* (*AËDES CALOPUS*), AND THE PRESENCE OF BACTERIA AND YEASTS.

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Introduction.

The clearing action of mosquito larvae in turbid water and their scarcity or absence in clean water, is an old-established popular belief in mosquito ridden districts of both the old and new world. If the presence of certain species of *Anopheles* larvae in clear running water, where they doubtless feed on algae, be excepted, it is probable that this general observation is in the main correct. The fact that numbers of mosquito larvae are frequently present in the small collections of clear water which occur in the cut ends of bamboos, the axils of leaves, or the smaller rock or root pools, is an apparent but not a real contradiction of the correctness of this popular belief, because in these instances one of two possibilities may have occurred. Either the large number of dormant eggs which hatched when rain first filled the receptacle was sufficient to check and control bacterial or yeast development from the start, or, as is more likely in the case of root or rock pools, the turbidity due to bacteria or yeasts had been rapidly cleared as the rapacity of the quickly growing larvae increased beyond the source of nutriment.

Mitchell (1907), referring to *Stegomyia fasciata*, says it is pre-eminently a bacteria-eating "wriggler," presumably on the strength of the known fact that the larvae develop rapidly in sewage-contaminated water.

Howard, Dyar, and Knab (1912) mention bacteria as forming food for mosquito larvae in general terms, stating that the spores of algae, particles of dust, bacteria, protozoa, minute aquatic animals of many different kinds are swallowed. They specifically mention that the

larvae of *Aëdes* are largely bottom feeders and may be seen vigorously working over dead leaves and vegetable débris with their mouth organs, apparently removing the fungoid growth which covers them.

Boyce and Lewis (1910) showed that the presence of mosquito larvae added to clean water led to an increase in the number of bacteria. Unfortunately the authors have not put on record the methods they pursued in arriving at this result, consequently we are left in doubt as to whether certain very necessary precautions to avoid fallacies were taken or not. For instance it is not stated in what stage or from what source the larvae they added to the clean water were taken, while, unless these factors were carefully controlled, there would be (as pointed out by the authors of the Carnegie Monograph on the Mosquitoes of North and Central America 1912) grave danger of the introduction of organic matter voided from the larval gut. The fact that an increase of bacteria followed the introduction of the larvae is therefore no valid argument against the popular belief of the clearing action of the mosquito larvae, unless the larvae had been introduced immediately after hatching, or just after a moult; the hatching of the eggs or moulting of the larvae having taken place in comparatively large quantities of clean water.

Bacot (1916) carried out a number of carefully planned experiments in Freetown, Sierra Leone, using newly hatched larvae of *S. fasciata*, with a view to ascertaining the amount and nature of the food consumed by the larvae and whether bacterial development in the breeding pans was a factor in the problem. He found that there existed a very definite relation between the development of bacteria and the growth of the larvae. Subsequently, on his return to England, he discovered, as detailed in a footnote to his report (see Experiments I and II), that water highly charged with organic matter, and swarming with bacteria, exerted a powerful stimulus to hatching on eggs which had not responded to immersion in clean water. The suggestion in his report that the bacteria themselves formed food for the larvae was based on the clearing action they displayed in water, rendered turbid by its enormous bacterial content, in conjunction with the fact that the gut-contents of larvae, taken from this water, showed only a few bacteria per field in contradistinction to the crowded fields displayed when water in which the larvae swam was examined. It being deemed impossible that bacteria could be excluded when the larvae were sweeping small particles of matter into their mouths, the explanation of their scarcity must therefore depend upon their rapid digestion.

A few rough experiments in which masses of bacteria and yeasts scraped from the surface of agar cultures were dropped into water containing larvae, showed that the larvae congregated round and greedily devoured them. Their jaws were evidently used to tear and disrupt the clumps of organisms, while the turbid cloud which formed round the struggling crowd of larvae was rapidly dissipated, presumably by the sweeping action of their mouth brushes.

Before describing the experiments and discussing their results, some preliminary explanations are necessary. For the sake of those readers who have no practical knowledge of mosquito breeding it is necessary to state that the conditions favourable for rearing the adults, though simple, require some nicety of adjustment for success, when the breeding pans are small. The essential points are temperature, the amount of nutriment in relation to the quantity of water, and the number of larvae in relation to both the latter factors. The temperature throughout the experiments under sterile conditions was 75° F. which if not quite high enough to give the speediest passage from egg to adult, is quite favourable for the species.

The amount of nutriment and number of larvae in proportion to the quantity of water were, however, in some of our experiments highly artificial.

In Bacot's report (1916) referred to above, the adjustment of these factors is dealt with from the aspect of the rearing of healthy adults. With the present research such careful adjustment was often neither possible nor necessary, the actual number of adults reared being altogether of secondary importance to the hatching of dormant eggs, and the relative speed of larval growth. Healthy conditions for the larvae occur when they are able to hold bacterial multiplication in check, when they do this very thoroughly their progress is slow and steady, if they hold it slightly in check their development is more rapid, while in cases where the balance of forces is delicate, larval growth is apt to be very rapid, resulting in a life or death race between them and the growth of bacteria. It mattered but very little if this balance, which could not in many cases be readily foreseen, resulted in the destruction of the larvae before the completion of their development owing to the exuberance of bacterial growth. While, although the lagging or eventual starvation of the larvae from lack of sufficient nourishment in some of the more narrowly adjusted experiments is regrettable, it need not lead to any confusion so long as it is borne in mind that the comparison of speed in growth must be made between the larvae in tubes of the same

series and not with those in some other experiments in which the conditions or media differed.

With regard to the hatching of eggs, much preliminary work was done by Bacot in Sierra Leone which is fully detailed in his report (1916), but for the benefit of those who have not had any opportunity of perusing it, the main facts may be briefly recapitulated as follows:

The eggs of *Stegomyia fasciata* are small black spindle-shaped objects of 0·630 to 0·650 mm. in length, by about 0·160 mm. in width if of average size. The shell, which is highly chitinized, is covered with a delicate cellular reticulation and from the central area of a large number, if not of all, the cell spaces rise small rounded bosses, which sections of the egg show to be gelatinous, and not merely the bulgings of the chitin wall of the egg. The eggs are deposited by the females singly, either on the water surface or the wet margins of objects in or surrounding the water surface; no doubt capillary action is responsible for the stranding of many eggs. Incubation is complete after a period of 30 to 50 hours according to the temperature, and Bacot states that it is necessary that the surface of the egg be kept moist while incubation is in progress. McGregor (1916) seems also to have experienced this necessity. After incubation the eggs now containing living larvae may be dried and remain dry for many months, without losing their vitality. Hatching on immersion, after incubation and drying, is generally erratic, a few eggs or a number may hatch within a few minutes; some will take hours, while others will remain dormant under water for days, weeks, and even months, and yet eventually yield healthy larvae.

The *quantity* of water in which the eggs are immersed makes no appreciable difference, nor does the age of the female, or whether the eggs were of a late or early batch. Changing the water in which the eggs were laid had no effect when the fresh water was from the same source and of the same temperature, but a fall in the temperature of the water in which the eggs were lying, exposure to cool air for a few minutes followed by reimmersion in the same water acted as a stimulus to the hatching of a varied percentage of the dormant eggs. The effect of cooling, however, seldom caused more than a small percentage of the dormant eggs to hatch and sometimes had no effect. The immersion of incubated eggs before they had been allowed to dry and others of the same batch after drying, gave divergent results[1].

[1] Although we have not yet followed this point up by planned experiments we have some reasons for supposing that the divergence may be due to bacterial action, during or immediately following incubation.

Preliminary Notes.

The experiments on which this paper is based were carried out in intervals of time during the course of other work, which in view of the war was of more immediate importance, although the actual delay which resulted from this cause was not serious, because, owing to the slow growth of larvae in some experiments, and the lengthy periods during which eggs remained dormant in others, slow progress was inevitable. From another aspect, however, the length of the gaps, extending in some instances over several months, between an initiatory experiment and its repetition, exerted a considerable influence on the final results, introducing an element of uncertainty as to the nature of the cause to which divergent results were due. For instance, the action of killed cultures and sterile extracts on dormant eggs is not consistent (in an earlier and later series of experiments) and it remains a matter of opinion whether the contradictory nature of the results arises from the treatment of the eggs, the conditions to which the parents were exposed, or the fluctuations in their heredity, which is possibly adjusted to the needs of climatic conditions in the natural habitat of the race of *S. fasciata* experimented with.

The bacterial side of the research was greatly hampered in that one of us had to drop all practical help in research owing to war work at a distance from the Institute, and, although he continued to act in an advisory capacity, it was not possible in his absence to follow various interesting points or to determine the species of the bacteria and yeasts which appeared in the course of the experiments.

It is perhaps necessary to add a word of explanation in regard to the use of the word "sterility." When working with a definite species or group of bacteria for which the suitable conditions of temperature and description of media are charted, the word "sterility" has a definiteness which hardly applies to this work. As here used the word refers to a failure of the organisms to make their presence apparent under circumstances conditioned to the needs of the mosquito larvae. Cultural tests for sterility were carried out with tubes of peptone broth and agar slopes for bacteria; in the case of yeasts with tubes of wort agar. In many cases experimental tubes which repeatedly gave sterile results when the inoculation was performed with a platinum loop were proved to be infected when the test was carried out with a Pasteur pipette which transferred 0·25 to 0·5 c.c., showing how slight the infection really was, either owing to the unsuitability of the temperature or

medium for the organism, or more probably to the clearing action of the larvae. In other cases when infection was suspected owing to the progress of larvae in comparison with their fellows which had hatched from eggs of the same batch in other tubes, it was only proved when cultivations on agar were made with quantities of 0·25 to 0·5 c.c. and the tubes were incubated at 60° to 75° F. It is further significant that larvae from the same batch of eggs might in one tube make no progress at all while in a similar tube perceptible progress would take place, only to cease before the third instar was attained, raising a suspicion of the presence of an infecting organism which either died out or was killed off by the larvae, which subsequently starved. These facts suggested that when eggs hatch the conditions are seldom if ever *really sterile*, but that the organism responsible for the stimulus is unable to colonize the tube owing to small numbers or want of adjustment between itself and the conditions. On the basis of this suggestion the difference between hatching and no growth on the part of the larvae and slight growth preceding death would be explained by the yeast or bacterial infection, probably due to infection from the eggs, dying out immediately in the former instance and more gradually in the latter, possibly owing to the action of the larvae.

Nevertheless, in spite of our frequently correct suspicion, that larval growth indicated infection, there were a few cases in which adult insects were reared under sterile conditions—the final tests being to transfer the dead mosquito from the tube in which it had been reared and died to a tube of peptone broth in which it was allowed to remain for a week or more under a variety of conditions of temperature. In these cases only one or two adults resulted from several larvae, and the possibility remains that the real source of nutriment was obtained from their dead comrades. This last surmise is, however, at issue with the fact that the presence of dead larvae did not in most cases enable the living survivors to complete their development.

We desire to take this opportunity of recording our thanks and indebtedness to our colleagues on the Staff of the Lister Institute for their valuable suggestions and advice, especially to Dr Harriette Chick and Professor Harden, without whose assistance many of the experiments could not have been carried out.

Methods.

Sterilization. One section of the experiments with eggs and larvae was carried out with unsterilized eggs, and larvae newly hatched from eggs put into clean water which were invariably found to be infected. In the other section attempts were always made to render the eggs sterile before use, and in spite of a number of probably unavoidable failures experience showed that with careful precautions it is quite possible to work under sterile conditions.

The following methods for sterilizing eggs were employed:

(*a*) Eggs were placed in a minute cotton bag, dipped into 2 % lysol for 5–10 minutes and washed in boiled water. The bottom of the bag was then snipped off and allowed to fall into a tube of broth.

This method gave a few successful results, in many cases, however, sterility broke down.

(*b*) Eggs were washed in warm (about 90° F.) soft soap and water for 15 minutes, then in 2 % lysol for 5–10 minutes; in boiled water for 5 minutes and then transferred by Pasteur pipette to tubes containing the sterile water or media.

This method gave many successful results, but there were some breakdowns in sterility, chiefly due to moulds and sporing bacteria.

(*c*) Eggs were washed in tap water by using a fine jet in a deep pan of water; they were then pipetted into weak lysol, about 0·5 %, and vigorously washed round by a jet; after a few minutes they were transferred to 2 % lysol and the stirring by jet repeated. After this they were again placed in 0·5 % lysol and then into boiled water.

Methods (*b*) and (*c*) were varied in minor detail from time to time.

(*d*) A method of sterilization by formalin vapour was tried, but it was too drastically applied in the first instance, the larvae dying on emergence from the egg. Although after suitable development it would possibly have given favourable results, it was not proceeded with, owing to the successful results obtained with (*c*).

Towards the close of the experiments it was found that the work of sterilization was rendered more certain, and less elaboration of the sterilizing process was necessary if more care was bestowed on obtaining eggs less liable to be covered with a number of species of bacteria, more especially those forming spores. Arrangements were therefore made to rear the parents from which it was proposed to obtain eggs for experiment under conditions which rendered the presence of an infection of sporing bacteria and moulds less likely. A breeding pan

was carefully cleaned and a number of larvae were washed in five changes of tap water, by thorough agitation and violent currents set up by a water-jet; there is little danger of the larvae being washed out of a deep pan, as they invariably keep at the bottom when the water is agitated. They were then left 24 hours, so that they might void the contents of their guts, and then again washed in two or three changes of water. After this preliminary treatment they were put into the clean pan and fed exclusively on broth cultures of *Bacillus coli*. As a result of this procedure the percentage of breakdowns in the sterility of the experimental tubes was much reduced.

One or two precautions with regard to pipetting the eggs are also advisable. Floating eggs should be avoided, and single detached eggs alone should be taken up. When two or more eggs remain attached after immersion in the lysol there is a probability that at the point of attachment a portion of the surfaces will not have been completely sterilized. The reason for avoiding floating eggs is also the danger of incomplete sterilization. In order to get rid of eggs attached to each other, the eggs may be sifted through motor veil gauze or any other very fine gauze.

The whole of the experiments are placed as an appendix, references to them only being given in the text.

Section A. Eggs.

Effect of contaminated water. Unsterile conditions.

Experiments I and II, already referred to on page 483, show clearly the powerful action of water charged with organic matter and living organisms on dormant eggs which failed to hatch in clean water.

Effect of alkaline solutions—under unsterile and sterile conditions.

Experiments III and IV. It was suggested by one of our colleagues that the results which followed the addition of water or other media charged with bacteria or yeasts might be due to the ammonia content. Aggramonte showed (1902) that the presence of lye from wood ashes stimulated the early hatching of the eggs. Two trials were carried out, one under unsterile and the other under sterile conditions. From these it seems quite clear that, although a few eggs may respond to the presence of alkaline solutions in either tap or distilled water, the effect is very feeble in comparison with that due to either contaminated water or a broth culture of *B. coli*.

Effect of acidity—sterile conditions.

Experiment IX. Early in the course of the experiments the possibility of the effects resulting from the addition of living bacteria to media such as peptone water or peptone broth being due to acidity was considered. A dilute solution of HCl adjusted to give an acid reaction similar to that in a tube in which the growth of bacteria caused the eggs to hatch produced no response during three days while the eggs were lying dormant in tubes of peptone water and peptone broth, but within 18 hours of the inoculation of these tubes with bacteria all the eggs hatched. A further trial with another tube of peptone water, belonging to the same series, in which the eggs had been dormant for 8 days, also showed a negative result during a period of 48 hours. The acid addition to this tube was increased so as to cause a much more marked reaction than that in the first trial. After inoculation with bacteria one egg hatched within 18 hours and another on the following day, and the remaining two on subsequent days. This halting response was apparently due to the slow and feeble development of the bacteria, owing to the extreme acidity of the tube. The larvae in the tube made relatively very slow progress.

We conclude from the results of the five experiments that the marked response of the eggs was due to the presence of bacteria or their products, apart from either the acidity or alkalinity produced.

Effect of introduction of different living bacteria into tubes of sterile media such as peptone water in which the eggs are lying dormant.

Staphylococcus pyogenes aureus. *Experiment IX* (page 506). A tube of peptone water in which eggs had been lying dormant for 15 days was inoculated from a culture of *S. aureus*. The bacteria failed to grow and the eggs did not hatch. Two days later the inoculation was repeated from the same culture, this time with success and the eggs all hatched within 16 hours.

Bacillus coli communis. *Experiment XI* (page 508). A tube of peptone water in which eggs had remained dormant for 11 days was inoculated from a culture of *B. coli*. All the eggs hatched within 18 hours.

Experiment XX. In a tube of sterile broth, made from dead insects, only one egg out of a number hatched. On the 34th day a quantity equal to about 1 in 7 of filtrate from a broth culture of *B. coli* was added. This had no effect on the dormant eggs, and the tube on

the 39th day was inoculated from a living culture of *B. coli*. The dormant eggs hatched within a few hours. A similar result followed the inoculation of a tube of peptone broth in the same experiment with *B. coli*, the eggs hatching within a few hours of the introduction of the bacteria after they had lain dormant for 39 days.

In Experiment XXIII several instances occur. In a sterile tube of distilled water containing 1 part in 6 of a filtrate from a broth culture of *B. coli*, eggs that had been dormant for 28 days hatched within 12 hours after the tube had been inoculated from a living culture of *B. coli*.

In a sterile tube of distilled water containing 1 portion in 6 of beef broth the eggs hatched on the 50th day within a few hours after the inoculation of the tube with *B. coli*. A similar tube was infected on the 60th day with a small quantity of a broth culture of *B. coli*; the eggs hatched within 10 to 15 minutes. On the 100th day of the experiment eggs that had been lying dormant in a tube of sterile distilled water hatched within 10 minutes of the addition of 3 % of a living culture of *B. coli*.

In a duplicate tube the addition of 3 % of a recently killed culture of *B. coli* caused 1 egg to hatch out of 11 within 15 to 20 minutes, but no more hatched within an hour and a half, when the addition of 3 % of a living broth culture of *B. coli* caused 6 eggs out of the remaining 10 to hatch within 15 minutes. The remaining eggs did not hatch at all and were most probably dead.

Unidentified bacteria.

Cases in which hatching followed the breakdown of sterility were frequent; instances will be found in most of the experiments, in many cases two or more tubes containing different media being affected. Although the species of bacteria were not identified it was clear from their behaviour when sub-cultured that they were of different kinds; some developed spores, while at least three different species were present, which would only grow at a temperature below 80° F.

Apparently any species of living bacterium may act as a stimulus though there is some evidence that certain species act more quickly than others (see Experiment XXIV). It is also significant that hatching only follows inoculation with a small quantity on a loop after several hours, whereas if as large a quantity as 2 % or 3 % of a broth culture is added, hatching follows as a rule within 15 minutes. There is also some evidence which suggests that fresh cultures are more effective than older ones of two or three weeks' age.

Effect of the introduction of living yeast cells.

Experiment XI. To a tube of peptone water in which eggs had been lying dormant for 15 days, about 1 in 5 of a sterile watery extract of brewers' yeast was added. No result followed within one week, when the tube was inoculated from a living yeast culture. All the eggs hatched within a few hours.

Experiment XXII. Some of the eggs placed in a sterile tube of distilled water, with 1 in 6 of beef broth added, remained dormant for 28 days. A small mass of cells from a culture of *Saccharomyces cerevisiae* on wort agar was added, when the dormant eggs hatched within a few minutes.

In the same experiment 7 tubes of distilled water, in which the eggs had lain dormant for 28 days, were subjected to the same treatment, most of the eggs hatched within 5 to 10 minutes.

Experiment XXIII. Eggs that had been lying dormant for 28 days in a tube of sterile distilled water, containing 1 in 6 of the filtrate from a broth culture of *B. coli*, hatched within 10 minutes of the introduction of a small mass of living cells of brewers' yeast.

In the same experiment (XXIII) on the 100th day 3 % of sterile autolyzed yeast was added to a tube of sterile distilled water in which there were dormant eggs. One egg out of 9 hatched within 15 to 20 minutes; after an hour and a half, during which period no further eggs hatched, a small mass of living yeast cells from a wort agar culture was added, and the remaining 8 eggs hatched within 15 minutes.

These examples afford clear evidence of the stimulus to hatching exerted by living yeast cells. In Experiment XI the yeast used was one that had been isolated from a human throat, but in the other tests *S. cerevisiae* was used.

Effect of the presence of living moulds.

No special tests were carried out with moulds, but during the course of the experiments it was remarked that when a mould developed in an otherwise sterile tube many if not all the dormant eggs hatched. It is apparently possible for moulds to exert this influence when very small and quite inconspicuous. In a number of cases in which eggs had lain dormant for several days their hatching was quite inexplicable until a tiny white speck, which proved subsequently to be a growing mould, was noticed. A microscopic examination in one such instance showed the hyphae ramifying over the surface of the egg shells. Many ex-

amples of hatching in apparent response to moulds occurred in Experiment XXII.

The most plausible suggestion as to the nature of the stimulus which induces the eggs to hatch would seem to be that of *a scent* which penetrates to the larvae lying dormant within the egg shells causing them to make vigorous movements resulting in the uncapping of the egg. There is, however, a difficulty in the way of the acceptance of this theory, owing to the partial or entire failure in some cases of killed cultures or filtrates of *B. coli* and sterile yeast extracts to bring about the hatching of the eggs. This subject is dealt with in greater detail in the following section.

The effect of killed bacterial cultures; sterile filtrates of B. coli *and extracts of brewers' yeast.*

Until nearly the close of the experimental work no results had been obtained from the addition of killed cultures and sterile filtrates of *B. coli* (see Experiments XIV, XXI and XII). On one occasion (Experiment IX) the addition of about 17 % to 20 % of a sterile autolyzed extract of brewers' yeast caused eggs which had been lying dormant in a tube of peptone water for a month to hatch within one hour, but the larvae died within a few minutes. In Experiment XI about 8 % of a sterile watery extract of brewers' yeast was added on the 15th day to a tube of peptone water containing dormant eggs, but no action having resulted within a week, the tube was inoculated from the culture of a species of yeast isolated from a human throat. The eggs hatched during the following night.

When sterilized eggs were pipetted into tubes of distilled water containing 2 % or 3 % of an autolyzed extract of brewers' yeast (Experiments XVI and XVII) hatching was not general, nor did hatching occur any more freely than in different media in the other tubes. In Experiment XVII larvae continued to hatch out from dormant eggs over long periods. In one case eggs that had remained dormant for over 90 days hatched without any interference with the tube. While in another instance eggs that had been resting for 126 days hatched on the addition of boiled distilled water to three tubes which were almost empty owing to evaporation.

Experiment XXI. None of the eggs in two tubes of an autolyzed extract of brewers' yeast solution (3 % in distilled water) hatched within 96 hours, but one larva emerged in each tube within 120 hours. In one tube no more eggs hatched, but in the other two or three larvae

emerged and died before the 12th day; both tubes were then inoculated from a living culture of *S. cerevisiae* and all the remaining eggs hatched during the night.

In spite of the foregoing negative evidence we thought it desirable to test the matter further, because it seemed possible that the failure might be due to either too high a temperature in the sterilization process or to the yeast extract not being sufficiently fresh—some days having been allowed to elapse between preparation and use.

With this object Experiment XXIV was planned. A number of tubes of distilled water containing eggs were used on the fifth day after the addition of the eggs. Cultures of *B. coli*, *S. aureus* and autolyzed extract of brewers' yeast which had been autoclaved (120° C.) were used, also cultures of *B. coli* and *S. aureus* which had been killed by steam heating at 100° C., a culture of *B. coli* killed by chloroform and a sterile filtrate of *B. coli*. The addition to the tubes was 6 %, except that in one of the tubes only 2½ % of the yeast extract was added, but this alteration made no difference to the result. It will be seen that in contradistinction to the earlier results the sterilized cultures and yeast extract were effective, but not the filtrate. This last was several weeks old at the time of use, but fresh filtrate had been used in the earlier trials without effect. It is interesting to note that the eggs were slower in responding to the *S. aureus* than to *B. coli* in all four tubes.

The results were so generally at variance with those in the earlier trials that it seemed probable that the difference must lie in the eggs. With a view to testing this hypothesis advantage was taken of several tubes of distilled water in Experiment XXIII in which eggs had been lying dormant for 100 days. These were tested by adding a quantity equal to about 3 % of a living culture of *B. coli*, autolyzed yeast sterilized by autoclave and *B. coli* sterilized by autoclave, the last two being from the same tubes as the additions to the tubes in Experiment XXIV. It will be seen that the results again differ, being more in line with the early trials, but not quite in conformity. There seems no doubt but that the susceptibility of the eggs varies greatly while it is also consistent with the results to suppose that the cultures but not the filtrate used—possibly because of their freshness—were more effective.

A further trial, Experiment XXV, was carried out to test the watery extract of yeast as against an autolyzed one, and both as against living cells from a culture of *Saccharomyces cerevisiae* on wort agar.

As a whole this test shows clearly that while, for these particular eggs, the autolyzed extract and the living cells were almost equally

effective, the watery extract was far less so, and in one tube had no effect at all within three-quarters of an hour. The difference in the results in the several tubes must be taken as measure of the variable susceptibility of the eggs. It will be noted that in one of the tubes to which the *living yeast cells* were added, there was only a partial response within the first three-quarters of an hour. It is regrettable that no tubes containing eggs which had long lain dormant were available to control results, as was done with the previous trial (Experiment XXIV).

In discussing these results it is necessary to note that hatching may follow if during the sterilizing process the eggs are left too long in lysol. Sometimes these eggs do not uncap until after their transference to water, but in both cases the larvae fail to come right out of the shell. The same phenomena followed when eggs were exposed to formalin vapour for several hours. After transference to water large numbers uncapped, but the larvae only partially came out of the shells. In these instances no movement on the part of the larvae was ever observed. It is possible that they were killed within the egg, but cut or partially cut the cap in their dying struggles. There is a difficulty in the way of the acceptance of this explanation, however, because it is not easy to understand why, in the case of those placed in lysol, the uncapping should sometimes be delayed until after they were transferred to water.

The differential hatching of the eggs may, as pointed out in the introductory notes, arise as an hereditary trait owing to an adaptation to meet the needs of seasonal changes. On the other hand there is evidence that treatment during incubation or prior to full immersion may be the cause of more ready response to immersion. Bacot (1916) found when in Freetown that of a given batch of eggs, which had incubated on a wet surface, 98 % of those which were immersed without allowing them to dry hatched within 30 minutes; of a portion of the same batch placed in water after 24 hours' drying the hatching of 84 % was distributed over a period of 65 days; while the balance of the batch, which was kept dry for 7 days before immersion, again showed a divergent result, 43 % hatching within 24 hours, another 11 % following within 51 days and of the balance 8 % containing living larvae when dissected after 81 days.

In Experiment XVI the eggs were not allowed to dry before sterilization, as it was desired to test the theory that a check in the influence of bacterial action on the eggs might be the cause of delay in their hatching when subsequently immersed. Although the larvae

did as a matter of fact emerge rather freely from these eggs under sterile conditions, the evidence from Experiment XXIII, in which also they were not allowed to dry before sterilization, is entirely negative, the number hatching under sterile conditions being small; while in Experiment XXV in which dried eggs were used the percentage hatching in some of the tubes was considerable. It is probable that the problem is a complex one, two or more factors being concerned.

The results of the experiments on eggs is so far clear and decisive in regard to the fact that the presence of bacteria, yeasts and, less definitely, moulds, does exert a stimulus causing eggs to hatch that would, apart from their presence, have remained dormant for a longer period. It also seems definite that this stimulus is less powerful, or may be altogether ineffective, if killed cultures or sterile filtration and extracts of bacteria or yeasts are used. The difference is apparently one of *quantity* not *quality*, its extent being chiefly dependent upon some variation in the susceptibility of the eggs.

Section B. Larvae.

Unsterile conditions.

The fact that the larvae greedily consume clumps of bacteria or yeasts (*S. cerevisiae*) removed from agar cultures has already been mentioned in the introductory remarks. This rough test is open to the objection that what the larvae struggled for was not the organism but the organic matter on which they were living, or by-products produced in their development.

In Experiments V and VI, *B. coli* washed by centrifuging were given as food to a batch of larvae, while to an equal number in a similar quantity of water no food was supplied; the unfed batches constituting controls. The results show conclusively that the larvae cannot only live, but thrive on the bodies of bacteria.

Sterile conditions.

These early trials, followed by a series of experiments (VII, VIII, IX and XI), afford examples, in which the eggs were sterilized and transferred by Pasteur pipette into tubes containing various sterile media, the object being to ascertain if it was possible for the larvae to develop under sterile conditions. In the main the results are clear and decisive; while sterility is maintained, larval development is either inhibited or proceeds excessively slowly in conjunction with a high, or

more frequently a total mortality. The infection of a tube with bacteria is generally followed by larval progress, usually rapid. The only exceptions are cases in which the larvae had existed under sterile conditions for long periods and seemed incapable of adjusting themselves to the change. Exceptions to the general rules of rapid larval development in infected tubes are most noticeable in Experiments XII and XIII when the bacteria which caused the breakdown in sterility proved to be feebly growing species, which altogether failed to keep pace with the larval requirements. In Experiments XI, XVI and XVII adults were reared under sterile conditions, the greatest success (in Experiments XI and XVI) being attained in a medium of pure beef broth (without the addition of salt or peptone) which for some unexplained reason seemed favourable for slow but sustained growth. Two adults were reared in separate tubes of a 3 % solution of an autolyzed extract of brewers' yeast in distilled water (Experiment XVII). One of these was reared under sterile conditions and the other ought, properly speaking, to be credited to these conditions as, although a mould was present in the tube when the adult emerged, all the evidence is against any suggestion that the larvae obtained any nutriment from this source —the presence of moulds being seemingly inimical or neutral to larval development; in this particular instance all the remaining larvae in the tube died.

While in these, and in fact in all, cases of larval progress under what are apparently sterile conditions, the possibility must not be excluded of a slight infection occurring during a portion of the larval existence and then dying out, the only evidence in support of such a supposition is the fact that larval progress varied in what were apparently sterile tubes independently of the media. This has happened in one or more out of a batch of tubes of the same medium, the eggs being part of a number all sterilized together. Taking into consideration the variability exhibited by the eggs at a stage when they contain fully developed larvae, it will require a considerable body of positive evidence based on carefully planned experiments before such a possibility can be accepted as a probability.

In Experiment XI a test was carried out to see if a change from one sterile medium to another had any effect in stimulating larval growth. Of two larvae withdrawn on the 15th day from a sterile tube of peptone broth, one was transferred to a tube of broth made from dead insects and the other to a tube of water in which horse dung had been steeped. After a pause of some 48 hours some evidence of quickened growth was

observed, but the progress was not continuous and both larvae died some 50 days later without having completed their larval development.

Experiments XII and XIII were designed with a view to trying sterile media composed of substances more likely to be met with by mosquito larvae under natural conditions than those normally used for bacteriological purposes. Tubes containing water in which horse dung had been steeped 48 hours previously and a broth made from dead insects—chiefly flies—were used. The series afforded but little evidence bearing on the original purpose of the tests owing to the infection of the tubes by obscure species of bacteria whose presence was difficult to detect, and such evidence as was forthcoming was negative. The tests convinced us, however, of the unsuitability of these media which were likely, owing to the sources from which the nutriment contained in them was drawn, to harbour sporing bacteria.

Particulate matter. One of our colleagues suggested the possibility of the necessity of particulate matter in the medium as it seemed possible that larvae swimming in a true fluid might be unable to pass it through the alimentary system in the absence of solid particles in the intestine. We accordingly added powdered animal charcoal to a number of tubes of a solution of autolyzed yeast in distilled water (Experiments XVI and XVII) with apparent success at the outset, as the larvae not only ingested it—the dark gut content being clearly visible—but made quicker progress than others in the same media without the charcoal. The advantage did not, however, continue and, although one reached the pupal stage, no adults were reared; on the other hand, as already recorded, one adult was reared from a sterile tube of the yeast extract solution, without the charcoal. It is also to be noted that the insect broth which contained much solid matter also failed, under sterile conditions, to confer any advantage after the first few days.

A few trials were carried out to test if milk acidified to such a degree that the colloidal particles of the casein were increased in size but not precipitated and white of egg precipitated as very minute particles, would afford the necessary pabulum for the larvae. After sterilization small quantities of these preparations were added to media in which eggs had already hatched, but in which the larvae were making no progress, or the larvae were transferred by Pasteur pipette to tubes containing milk or white of egg in this form. Examples will be found in Experiments XIII, XVIII, XIX and XX. In no case did the addition or presence of the substances enable the larvae to develop

while sterility was maintained; although in some instances slight progress followed the addition (Experiment XIX, page 518), it soon ceased, the larvae eventually dying without attaining their full growth.

Killed cultures of B. coli.

In Experiment X a preliminary trial was made to ascertain if the larvae could be reared under sterile conditions upon a culture of *B. coli* killed by heat. No success followed, but young larvae hatched from unsterilized eggs made rapid progress in a control tube, this tube being of course infected by the larvae. Experiment XIV was a repetition on a larger scale; several tubes were prepared, but the eggs placed in these failed to hatch. A single newly hatched larva (the only one which could be spared at the moment) was transferred from another experiment to one of the tubes. It failed to make any progress during a month and died within a few hours of the inoculation of the tube at the end of this period.

Sterile filtrate of B. coli.

In Experiment XXIV a culture of *B. coli* killed by chloroform was added to tubes of sterile distilled water containing eggs; a number of these hatched and tube No. 12 remained sterile, although its fellow No. 11 became infected on the 11th day. None of the larvae in No. 12 passed the first moult, while some of those in the infected tube had made considerable progress and were in the third instar. The larvae in tube No. 12 all died in the second skin, whereas in the infected tube the larvae were well grown before the food supply failed, two at least having attained their fourth (last larval) instar.

Thinking that possibly the action of the heat used in killing the cultures of *B. coli* might have the effect of destroying its nutrition value for the mosquito-larvae, a number of trials were carried out with the sterile filtrate from broth cultures of *B. coli*. No success attended any of them, either in neat or diluted condition, nor did the addition of the filtrate to other sterile media in which larvae were living produce any effect, apart from the few cases in which sterility broke down at the period when the filtrate was added. (See Experiments XIX, XX and XXIII.)

Living yeast and yeast extracts.

See Experiments XVI, XVII and XXI; note also the conclusion of XXII, page 521, of XXIII and of XXIV and the conclusion of XXV. There is a break of similar character between the nutritive value of

living *S. cerevisiae* and sterile extracts of brewers' yeast to that which separates living from dead cultures, or sterile filtrates of bacteria. It is not quite so definite, however, as the larvae do make progress in solutions of autolyzed yeast extract, whereas throughout the whole course of the experiments no larval progress has occurred in sterilized cultures of bacteria or sterile filtrates. The reason for this difference is not obvious and it seems possible that by some system of concentrating and extracting bacterial cultures analogous to the autolyzing process with yeasts, the difference might be eliminated.

The effect of the presence of moulds on larval growth.

Although no experiments were planned with a view to testing the influence of moulds, their occurrence in both sterile and bacterially infected tubes was so frequent in some of the experiments that a considerable amount of general observation was possible. The impression gained during the progress of the research was that moulds were inimical to larval development, but a careful consideration and comparison of the notes show that the fatality which so frequently followed their appearance in the experimental tubes cannot be clearly demonstrated as due to their presence. In a few cases of rapidly spreading surface moulds it is extremely probable that they were an accessory, if not the actual cause of death, and in Experiment XXII an instance of mortality apparently due to moulds occurred in tubes Nos. 4 and 5 of the distilled water series. What does, however, appear quite definite is that they are of no service whatever to the larvae as a source of food supply. In fact it seems reasonable to suppose that they may compete with the larvae for whatever supply of nutriment is available. Although the evidence on this point is not altogether consistent, in Experiment XXII it appears quite negative, there were several instances in which fully grown larvae died in tubes where moulds had developed. In a few cases one or two out of several of the larvae succeeded in completing their development, the others dying rather inexplicably if the moulds were not implicated. Two or three instances occurred of moulds growing from the mouths of dead larvae, as though swallowed spores had developed in the alimentary canal; while there is of course no evidence that the development of the mould preceded death, the spores must presumably have been ingested by the living larvae. Notes referring to the presence of moulds will be found in Experiments XXI, XXII and towards the close of XVII and XXIII.

Our conclusion respecting the evidence as a whole may be stated as follows: that the larvae of *Stegomyia fasciata* greedily consume both bacteria and yeasts on which they can thrive in the absence of any other food, whereas in very many instances they fail entirely to develop on a variety of nutritive fluids and particles, including dead bacteria, under sterile conditions. In the far less numerous cases where progress was made under apparently sterile conditions, growth was always relatively very slow to that under otherwise equivalent but unsterile conditions, and the mortality was very high. The rearing of adults under sterile conditions being so exceptional we feel justified in stating that the presence of bacteria or yeasts is a practical necessity for the maintaining of the species. If one considers the facts shown by Bacot (1916) regarding the scarcity of bacteria in the alimentary canal of larvae taken from water swarming with bacteria in relation to the exclusively browsing habit of the young larvae and its partial retention by the older ones when feeding, together with the experimental results above recorded, there seems good reason for supposing that bacteria and yeasts afford the chief food supply of the larvae. The ingestion of larger particles and the structure of the jaws are not incompatible with this view, because such particles are likely to be covered with a bacterial growth while the jaws are of use in gnawing away portions of decaying organic matter. They were evidently used to disrupt clots of both bacteria and yeasts in the preliminary experiments mentioned in the introduction.

It seems probable that this knowledge may be of assistance in the destruction of this species of mosquito, as it should enable the methods now so largely available for the purification of water from bacteria to be utilized. On a minor issue it may be of service in enabling Sanitary Officers to escape the annoyance caused by the failure to breed more than one or two isolated species from jars containing mosquito larvae captured by their inspectors. This failure no doubt arises owing to starvation, because the bacteria are killed by the action of light, it being customary to stand jars with wrigglers in the full light of a window. There is the further possibility that the eggs after a careful and probably lengthy research might be found to be of service as a rough and ready means of testing the relative purity of water in regard to living organisms.

EXPERIMENTS WITH UNSTERILIZED EGGS.

Effect of foul water charged with bacteria.

Experiment I.

100 c.c. of tap water was put into each of two glass pans. The water temperature during the course of this experiment was about 65° to 67° F.

A batch of about 350 eggs laid on filter paper three weeks previously and allowed to dry after incubation, but stored in a moderately humid atmosphere, was divided into two lots, approximately half being immersed in each pan.

To the water in one pan (*A*) was added 3 c.c. of sewage water (a fragment of human faeces was placed in water and allowed to incubate for 48 hours at 80° F.); to the water in the other pan (*B*) nothing was added.

After	1 hour	in *A*	4 eggs had hatched.		
„	„	*B*	no eggs had hatched.		
After	4 hours	in *A*	10 additional eggs had hatched.		
„	„	*B*	no eggs had hatched.		
After	20 hours	in *A*	113 additional eggs had hatched.		
„	„	*B*	6 eggs had hatched.		
After	70 hours	in *A*	9 additional eggs had hatched.		Total 136.
„	„	*B*	36 „	„ „	Total 42.

Experiment II.

The experiment was repeated, using water in which horse dung had been steeped for the stimulating fluid. The quantities and other arrangements were similar to those in Experiment I, 3 c.c. of the contaminated water was added to pan *A* at the start, but none to pan *B*.

After	18 hours	in *A*	148 eggs had hatched.
„	„	*B*	11 „ „

3 c.c. of the contaminated water was then added to pan *B*. At the expiration of another 18 hours 159 additional eggs had hatched in pan *B*. The size of these larvae suggested that they must have hatched within an hour or two of the addition of the manure water.

Experiment III.

Contrasted effect of alkali and water charged with bacteria. Temperature 75° F.

Into each of 4 pans containing 100 c.c. of tap water was placed ·007 gr. of incubated eggs of *S. fasciata* (the number would be about 200 or 300 eggs). The pans were kept all night at 75° F. Pan (1) formed a control. To pan (2) was added a solution of washing soda, producing an equivalent of 1 in 1000 in the pan; to pan (3) sufficient ammonia was added to produce 1 in 1000. To pan (4) about 5 c.c. of peptone broth from an experimental tube in which a bacterial infection caused the eggs to hatch.

After 6 hours (No. 2) Soda, showed a few larvae out.
„ (No. 3) Ammonia, showed a few larvae out
„ (No. 4) Contaminated, many.
„ (No. 1) Control, only one or two.

To the control pan about 2 c.c. of an infusion from horse dung was added, and the pans were left all night.

18 hours later (No. 2) Soda, showed a small number out.
„ (No. 3) Ammonia, about the same number out.
„ (No. 4) Contaminated, swarms.
„ (No. 1) Control, after the addition of the infusion of horse manure many more had hatched than in either the soda or the ammonia containers, but not so many as in the contaminated pan.

10 c.c. of water from pan (No. 4) was added to pans Nos. 2 and 3. An examination 6 hours later showed that the numbers of larvae had increased by one-third to a half, the freshly emerged larvae being easily distinguished from those which had hatched some hours previously, by their pointed conical heads.

Experiment IV. *With sterilized eggs.*

Contrasted effect of alkali, filtrate of B. coli *and living* B. coli.

Tubes taken from Experiment XXI (see page 519).

A batch of eggs was sterilized by Method *b* and from 12 to 20 were pipetted into a number of tubes containing 10 c.c. of sterile distilled water and one (No. 5) containing sterile filtrate of *B. coli*. After 20 hours at 75° F. the tubes were examined and four containing distilled water were selected in which only one or two eggs had hatched, the idea being to use resistant eggs which did not respond without stimuli.

In tube No. 1, 2 eggs had hatched (1 larva was dead).
„ No. 2, 2 „ „
„ No. 3, 2 „ „
„ No. 4, 1 „ „
„ No. 5, 0 „ „

To No. 1 was added 1 c.c. of water in which horse dung had been steeped for 48 hours at 37° C. ... ... = 10 %.
„ No. 2 was added 1 c.c. of a sterile filtrate of a *B. coli* culture = 10 %.
„ No. 3 „ 1 c.c. of a 1 % solution of washing soda = 1–1000.
„ No. 4 „ 1 c.c. of a 1 % solution of ·880 ammonia = 1–1000.
No. 5, a tube of filtrates of *B. coli* culture, was inoculated from a living culture of *B. coli*.

Result after 1 hour:

	Increase in hatching
No. 1, more than 12 larvae out	10 or over
No. 2, 4 larvae out	2
No. 3, 3 „	1
No. 4, 1 „	0
No. 5, 0 „	0

After 24 hours:

	Increase in hatching
No. 1, more eggs had hatched—probably all.	
[1]No. 2, 8 larvae out	6
No. 3, 3 ,, ...	1
No. 4, 2 ,, ...	1
No. 5, 12 ,, ...	12

After 68 hours:

No. 1, all the eggs hatched, larvae have grown rapidly.
No. 2, 9 larvae out—slight progress.
No. 3, 4 ,, no progress.
No. 4, 2 ,, both dead.
No. 5, all (12) larvae out, have grown rapidly.

The strength of the soda and ammonia solution in tubes Nos. 3 and 4 was doubled to 1 in 500.

After 24 hours no further eggs had hatched.

To each tube, Nos. 2, 3 and 4, ·5 c.c. of a culture of *B. coli* was added.

One hour later: all the remaining eggs in tubes 2, 3 and 4 had hatched—

No. 2, 12 or 13 living larvae.
No. 3, 12 or 14 living larvae.
No. 4, 12 living larvae.

The larvae in No. 4 (ammonia 1 in 500) all died within 24 hours.

Two days later the larvae in tubes Nos. 2 and 3 had made very rapid progress and had caught up to those in tube No. 5 in the matter of growth. They actually passed them during the next day or so and then slowed down. The larvae in all these tubes came to a standstill within 10 days, having apparently exhausted their food supply.

Experiment V (*unsterilized conditions*).

Ability of the larvae to develop on a diet of bacteria. Temperature 75° F.

Bacillus coli was washed by centrifuging 4 times in succession and a small quantity of the precipitate placed in 30 c.c. of tap water with 5 newly-hatched larvae.

Control. 5 newly-hatched larvae of the same batch were placed in 30 c.c. of tap water, minus the *B. coli*.

The control larvae marked time with little or no progress; 1 larva died on the 6th day and 1 was feeble and died on the 8th day. By the 11th day 2 of the larvae passed the first moult, while a third larva died on the 12th day. After 17 days 1 larva attained to its third instar; both the larvae were living after the 30th day, but were dead by the 37th day. The larvae given *B. coli* grew rapidly; by the 3rd day 2 of the larvae had passed the second moult and the others were nearly full grown in their second instar.

[1] It is probable that tube No. 2 was already infected as numerous later tests of *B. coli* filtrate added to tubes in which dormant eggs were lying, showed that the filtrate did not stimulate hatching.

The supply of washed *B. coli* in the pan was renewed. By the 6th day all the larvae were in the fourth instar. One pupated on the 8th day, and the remainder had pupated by the 10th day. Two ♀♀ specimens emerged on the 12th day and two more ♀♀ and a ♂ by the 13th.

The pan was then restocked with 5 newly-hatched larvae, but no fresh food given. All the larvae were in their second instar by the 3rd day; they reached the third instar by the 7th day, having exhausted the food supply, and were still in the same stage 10 days later.

Experiment VI (*unsterilized conditions*).

Ability of larvae to develop on a diet of bacteria.

Temperature 75° F.

Into one of two beakers, each containing 200 c.c. of tap water, was placed sufficient of a washed culture of *B. coli* to cloud the water.

20 newly-hatched larvae were placed in each beaker, and card covers placed on top to exclude dust.

Control. No appreciable progress, beyond the expansion of the head, took place within a week, then 8 died within 24 hours. Seven days later only 6 of the 20 larvae were living, 3 of which had passed the first moult. After another week (21 days from start) these 6 were still living, and making slow progress.

After 75 days there were two survivors, which by this time were slender larvae in the third instar. A little water mixed with faeces and therefore containing bacteria was added to the pan. Rapid growth was noticeable by next day; 1 larva had pupated within 5 days, and by the 9th day—84 days after hatching—2 adults were bred.

The larvae given *B. coli* made rapid progress; all were in the third instar by the 4th day; 16 had pupated by the 9th day; 4 ♂♂ and 1 ♀ emerged on the 11th day and the remainder followed within a few days; the last, which had lagged in the larval stage, taking 16 days.

Experiments under sterile conditions in tubes containing nutrient media.

Experiment VII.

Eggs sterilized by Method *a* were pipetted into a tube of peptone broth, after a night at 75° F., the tube was allowed to cool to 60° F., and some of the eggs hatched. The larvae showed no sign of growth after 48 hours at 75° F. The tube was then infected with *B. coli*; within 18 hours at 75° F. the broth was cloudy and the increase in size of the larvae was very obvious.

A curious feature of the experiment was that small bubbles of gas which were liberated by the fermentative action of the bacteria, formed on empty eggs, fragments of egg-shell etc. lying at the bottom of the tube, floating them up to the surface, when, the bubble becoming detached, they sank to the bottom and the process was repeated. Similar bubbles formed at the apex of the syphon tubes of the larvae, causing them to float upwards in spite of their struggles to swim downwards. So regular and constant was this process that the larvae got but little time for undisturbed feeding, and their growth was slow in comparison with their rapid start.

After 4 days the formation of gas ceased and the larvae again commenced to grow rapidly, and within 3 days some had attained their third skin. The tube, however, became very foul and all the larvae were killed.

Experiment VIII.

Eggs sterilized by Method *a* were pipetted into a tube of peptone water and a tube of beef broth without either salt or peptone.

No eggs hatched in either tube in response to the conditions and lapse of time, which were successful in the first trial.

Broth. After 48 hours sterility broke down in the broth tube; many of the eggs hatched and the larvae made rapid progress, 4 days later some of the larvae passed their third moult, but others were much behind on the 24th day of the experiment; 22 days after hatching the first adult was reared and others followed, but there was a heavy death-rate, presumably owing to there being too gross a growth of bacteria in the tube.

Peptone water. This tube remained sterile and, in spite of warming and cooling, no eggs hatched until the 3rd day, when one larva emerged; others followed and 5 or 6 were seen by the 5th day. Growth was not quite inhibited, but hardly any progress had been made by the 4th day after hatching, the larvae being still in the first instar.

On the 8th day of the experiment, 4 days after the hatching of the eggs, sterility broke down, the tube becoming cloudy. The larvae emerged from the remaining unhatched eggs and rapid progress commenced.

The larvae made rapid growth at first, and, owing to their number or the less vigorous growth of the bacteria, the conditions in the tube remained healthy, the larvae keeping the clouding of the peptone water in check and finally clearing it; they then began to find a shortage of food and ceased to grow, finally dying on the 24th day of the experiment—the last to die having reached the third instar.

Experiment IX.

(Includes notes respecting the addition of acid on dormant eggs.)

Eggs were sterilized by Method *b* and pipetted into four tubes of peptone water and four tubes of broth, only 5 or 6 eggs being placed in each tube.

After 48 hours, 4 eggs had hatched in one of the tubes of broth (No. 1) and in this tube only had sterility broken down; a 5th larva emerged in this tube next day. A plate culture was made from this tube, and a sporing bacillus isolated. On the evening of the 3rd day from the start one of the broth (No. 2) and one of the peptone tubes (No. 3) in which no eggs had hatched, were inoculated from the tube which had become infected. By next morning 4 larvae had hatched in the broth and 1 in the peptone tube. None hatched within the next 2 or 3 days in the remaining sterile tubes. The following test was then made: to two of the sterile tubes—broth No. 4 and peptone No. 5—was added sufficient of a dilute solution of HCl to produce a similar acid reaction to the broth tube in which sterility broke down. No response took place within 20 hours, but the remaining eggs in the inoculated peptone water hatched within this period.

By the 7th day of the experiment the larvae in the broth tube in which sterility broke down were all in the fourth instar, while those in the inoculated broth tube

were in the second and those in the inoculated peptone water in the third instar. No eggs had hatched in the tubes to which acid had been added 3 days previously and the tubes were now inoculated from No. 1; within 18 hours all the eggs in them hatched.

Another trial was made, with dilute acid of higher concentration, in one of the peptone tubes (No. 6) in which the eggs had now lain dormant for 8 days; the acid reaction after the addition being much stronger than in the original broth tube (No. 1).

After 48 hours, during which period no larvae hatched, the third tube to which acid was added (No. 6) was inoculated from tube No. 1; by next morning (18 hours later) one egg had hatched, another larva emerged on the 2nd day, and 2 others on following days. The bacterial growth in this tube was feeble, presumably owing to its relatively strong acidity, while the larvae in it made very little progress.

On the 14th day of the experiment two eggs were taken by means of a Pasteur pipette from the remaining sterile broth tube (No. 7) and transferred to a tube of peptone broth; they lay unhatched in the tube of peptone broth for 8 days, when the tube was inoculated with *Staphylococcus aureus*; within 16 hours larvae had emerged from both the eggs. They died, however, within 3 days, apparently owing to the vigour of the bacterial growth.

On the 15th day of the experiment, the broth tube from which these eggs had been removed (No. 7) was inoculated with *S. aureus*. The culture did not grow and the eggs failed to hatch. Two days later the inoculation was repeated from the same culture; this time with success, and the eggs which had been lying in the broth for 17 days hatched within 16 hours.

On the 32nd day of the experiment 1 c.c. of sterile autolyzed extract of brewers' yeast (= 17 % to 20 %) was added to the remaining tube of peptone water (No. 8) in which the eggs had been lying dormant for a month. The eggs hatched within an hour, but the larvae died within a few minutes of their emergence.

History of the larvae in the above experiment.

Tube No. 1; eggs hatched in response to casual infection.

The larvae were in the fourth instar by the 7th day; 4 days later the first pupa was noted and 3 days later the first pair of adults emerged; 2 ♂♂ and 3 ♀♀ followed within the next day or two—15 days from hatching.

Tube No. 2 broth and No. 3 peptone eggs hatched on the 3rd day of experiment. The bacterial growth was more vigorous in the peptone water than in the pure beef broth. Four days after the hatching of the eggs the larvae in tube No. 2 were in their second instars, while those in tube No. 3 were already in the third skin. This rapid progress on the part of the larvae in tube No. 3 (peptone water) did not continue long; the dense growth of bacteria rendered the conditions unhealthy and 4 days later the larvae in tube No. 2 were ahead of those in tube No. 3, while the latter were beginning to die off.

On the 11th day from the hatching of the eggs all the larvae in tube No. 3 were dead, while two of those in tube No. 2 had pupated. The remaining larvae were full fed and the broth was bright and transparent, the number of bacteria having been reduced to a minimum either as a result of the larvae devouring them or organic matter in the broth necessary for bacterial development.

Tubes Nos. 4 and 5. Four days after hatching, the larvae had made good progress, those in the broth tube being well ahead, while by the following day the growth of bacteria in the peptone water had become exceedingly dense and one or two of the larvae were dead. By the 7th day after hatching 4 larvae in the broth tube had pupated, while the surviving larvae in the peptone water were still in their third instar. Five days later (12 days from hatching) all the larvae in the peptone water were dead, and a pair of adults had emerged from the pupae in the broth tube; the remaining pupae and larvae died.

Tube No. 6. These larvae made very slow progress; the growth of bacteria in the tube being feeble. By the 6th day after hatching they had only attained to their second instar. Five days later the larvae were still all living and had made steady progress; one pupated on the 13th day from hatching and 2 ♂♂ emerged on the 17th day; 2 ♀♀ following on the 18th and 20th days.

Tube No. 7 (broth). Four days after hatching only 2 of the 4 larvae were living; these had made rapid progress and were in their fourth instar; 2 ♂ specimens were bred on the 11th day from hatching.

Experiment X.

Washed B. coli *killed by heat as food for the larvae.*

A small quantity of washed *B. coli* was put into two tubes of distilled water which were sterilized by heat.

Into one tube a larva was transferred from a tube of peptone water that had remained sterile for several days; into the other several larvae that had just emerged from unsterilized eggs placed in tap water. The cloudiness which followed in this tube indicated that the larvae had infected it. The growth of these larvae was rapid; on the 5th day they were well grown in the third instar—the sterile larvae having made barely appreciable progress in the first instar.

The progress of the infected larvae slowed down gradually as the water cleared, but they continued healthy.

In the sterile tube the larvae progressed very slowly and died after moulting on the 9th day.

Experiment XI.

(Includes notes concerning the effect of sterile yeast extracts on dormant eggs and a change of diet, under sterile conditions, on the larvae.)

Five to 7 eggs sterilized by Method *b* were transferred by pipette into the following tubes: 4 of peptone broth; 3 of peptone water; 3 of beef broth (no salt or peptone); 1 in boiled tap water and 1 in boiled distilled water.

After 24 hours eggs left in the lysol used in the sterilizing process had hatched; the larvae were dead and many had failed to get free of the eggshell. Only one egg hatched out of a number that were transferred from the lysol to tap water. Several eggs out of a large number left in the flask of boiled water hatched. Some eggs also hatched in the tubes of peptone broth, broth tubes, and in one of the peptone water tubes, but none in the boiled tap water or boiled distilled water.

After 48 hours one larva hatched out in the boiled distilled water; none in the boiled tap water.

During the same period in peptone water two larvae came out of 6 eggs in one tube only.

Beef broth. Tube (No. 1), 1 out of 7 eggs
,, (No. 2), 3 ,, 7 ,,
,, (No. 3), 4 ,, 7 ,,

Peptone broth. Tube (No. 1), 5 ,, 6 eggs (1 larva dead)
,, (No. 2), 4 ,, 6 ,,
,, (No. 3), 2 ,, 5 ,,
,, (No. 4), 2 ,, 6 ,,

The growth of the larvae was negligible.

Three days later (5 days from start):

Boiled tap water. None had hatched.

Boiled distilled water. No progress by the larva.

Peptone water. No further hatching; no progress by the larvae already hatched.

Beef broth. Tube (No. 1), 5 hatched out of 7.
,, (No. 2), 6 ,, ,, 7.
,, (No. 3), 4 ,, ,, 7.

All the larvae were making slow progress.

Peptone broth. Tube (No. 1), 5 hatched out of 6 (2 larvae dead); slight progress.
,, (No. 2), 4 ,, ,, 6; slight progress.
,, (No. 3), 2 ,, ,, 5; very slight progress.
,, (No. 4), 2 ,, ,, 6; little, if any, progress.

On the 8th day from start:

Boiled distilled water. Larva still in first instar.

Peptone water. No change, the larvae still in first instar.

Beef broth. A few more eggs had hatched; the numbers are now:
Tube (No. 1), 6 hatched out of 7.
,, (No. 2), 7 ,, ,, 7.
,, (No. 3), 4 ,, ,, 7.

All the larvae show slight growth, most of them being in the second instar, but one has not passed its first moult.

Peptone broth. The living larvae are now:
Tube (No. 1), 4 out of 6 eggs.
,, (No. 2), 4 ,, 6 ,,
,, (No. 3), 2 ,, 5 ,,
,, (No. 4), 4 ,, 6 ,,

Slight growth was apparent, but none had passed the first moult.

On the 11th day from start:

One of the *peptone water* tubes in which none of the eggs had hatched was inoculated with *B. coli*; by next morning (18 hours later) all the eggs in this tube had hatched.

In tube No. 4 of the *peptone broth* tubes sterility had broken down, and there was a slight bacterial infection. In this tube only were the larvae making any progress; in all the other peptone tubes the larvae were marking time.

Beef broth. In two tubes (Nos. 1 and 2), the larvae were growing steadily, if slowly; in No. 3 progress was very slow.

On the 14th day:

The larva which hatched in the boiled distilled water died in its first instar.

Peptone water. One of the two larvae which hatched in the sterile tube was dead. In the *B. coli* infected tube the larvae had already passed the second moult (about 48 hours after hatching). In the third tube, which remained sterile, the eggs were still unhatched.

Peptone broth. Tube (No. 3), one of the two larvae was dead, the other had moulted; (No. 2), 3 larvae were in the second and 1 in its third instar, all small and ill-grown. In tube No. 1 another larva had hatched, but one of those which had emerged previously was dead; of the living 2 were in the second and 2 in the first instar. In the infected tube (No. 4) the larvae were making slow but steady progress in their second and third instars.

Beef broth. Tubes Nos. 1 and 2 had become infected and the larvae were progressing.

15th day:

Distilled water. A second larva had emerged in this tube.

Tap water. Eggs still dormant.

Peptone water. In the *B. coli* infected tube all the larvae had reached their fourth instar and one had died.

To the sterile tube in which no eggs had hatched ·5 c.c. of sterile watery extract of brewers' yeast was added. (Without result.)

Peptone broth. Tube (No. 1), the two first instar larvae were taken to test if change to a different media would affect growth. A note will be found at the end of this experiment (page 511), both the second instar larvae were dead; (Nos. 2 and 3), no change; (No. 4), the infected tube, the larvae were making slow progress.

Beef broth. The larvae in the infected tubes (Nos. 1 and 2) progress steadily; in No. 3 the progress is scarcely perceptible.

19th day:

Distilled water. The second larva which hatched was also dead.

Tap water. None of the eggs had hatched.

Peptone water. In the tube infected with *B. coli* all the larvae reached full growth and then died.

Of the larvae which hatched out in the sterile tube, 1 had died; the living ones made no progress.

The eggs in the tube to which sterile yeast extract was added had not hatched.

Peptone broth. (No. 1), no living larvae remained; (Nos. 2 and 3), the larvae all dead; (No. 4), growth was variable, the larvae which hatched prior to infection were ahead of the others.

Beef broth. Larvae in the sterile tube were making very slow progress; in the infected tubes they were growing steadily, though not rapidly.

22nd day:

Distilled water and *Tap water.* No change.

Peptone water. In the sterile tube the larvae made no progress. The yeast extract did not cause the eggs to hatch in the third tube; this tube was inoculated with a living yeast *and the eggs hatched during the night.*

24th day:

Tap water. The eggs lay dormant in this tube for 24 days; 1 c.c. of an autolyzed extract of brewers' yeast was added (about 20 %); the eggs hatched within 15 minutes; the larvae, however, died.

Quantities, up to about 10 %, of this sterile extract were added to the sterile tubes of peptone water and peptone broth in which unhatched eggs were lying, but no further larvae emerged, and the larvae which had already hatched in these tubes did not respond by growing.

28th day:

Peptone water tube (in which the eggs hatched on the 22nd day following an inoculation with a living yeast). The larvae showed slow and varied growth; they eventually died without pupating—the growth of yeast in the tube being presumably too feeble to supply sufficient nutrition.

The larvae in the sterile tube of peptone water to which sterile yeast extract had been added, lived on for a long while, making little or no progress. A number were still living 95 days after the start of the experiment, and one lingered on until the 113th day, when it was still in its second instar.

Beef broth. The larvae in the sterile tube progressed very slowly, sterility being maintained as shown by the sub-culture tests made at intervals. After 50 days a few adults were reared; these emerged at intervals over a period of 10 or 12 days, one, which fell into the broth and was drowned, was transferred with a platinum loop to a tube of sterile broth, but no growth ensued. A living specimen was fed on a carefully cleansed patch of skin on the arm, producing the same quickly passing reaction mark as do normally bred *S. fasciata* when they bite this individual. This observation suggests that Schaudin's opinion that the reaction following the bite of the mosquito is due to a ferment is possibly incorrect. An attempt to feed another specimen, reared subsequently under sterile conditions, on the arm of a lady, who showed a very marked reaction to the bites of *S. fasciata*, failed as the insect died without biting, although tested on several successive days.

Result of test carried out on larvae taken from Tube No. 1 *peptone broth on the* 15*th day.*

Two larvae were withdrawn by Pasteur pipette on the 15th day from one of the peptone broth tubes which had remained sterile.

One of these larvae was transferred to a tube of broth made from dried and powdered insects (mostly house flies), the other to a tube of water, in which horse dung had been steeped. Both these tubes had been autoclaved. The plan was to test if a change of diet under sterile conditions would affect growth. The larvae at first made no progress, but after 48 hours they began to show signs of slow growth, which continued for 30 to 40 days, by which time the larvae were little more than half grown. They then marked time during a period of 10 or 12 days and died without making any further progress.

Experiment XII.

This test was carried out with a view to deciding if sterilized media of a kind more likely to be encountered in nature than those prepared for bacteriological experiments would allow of the larvae developing.

For this purpose tubes of water in which horse dung had been steeped for 48 hours and of a broth made in hot water from dead insects were autoclaved. Both media when tested under unsterilized conditions had been found to afford excellent breeding results.

Eggs sterilized by Method *b* were transferred to the tubes by Pasteur pipette.

Insect broth. No eggs hatched within 18 hours, but a few larvae were out in tubes Nos. 1 and 2 after 48 hours. None of the eggs had hatched in tube No. 3. One or two larvae were out in this tube also, after 72 hours. It was significant, however, that a small mould was observed on a group of floating eggs in tube No. 3. (Subsequent experiments showed that the growth of a mould acted as a stimulant, causing eggs to hatch.)

During 5 days the larvae made slow but steady growth in tubes 1 and 2, but made no perceptible progress in No. 3.

Tests showed that sterility had broken down in tubes 1 and 2, a feebly growing species of bacteria being present when tested after 8 days.

The development of the insects was very slow, taking about 50 days. In tube No. 3 a larva was still living, being only in its third skin after a period of 65 days. This larva died subsequently.

There was some mortality in the late larval stage, but a few adults were reared in each of tubes 1 and 2.

Manure water. No eggs hatched within 18 hours.

A few larvae were out in one tube within 48 hours.

Larvae were out in all tubes within 72 hours.

During 5 days the larvae made slow but steady growth in all the tubes, and were in either the close of the second instar or the beginning of the third. Progress was slower in one tube than the others, but tests proved that all the tubes were infected with some feebly growing bacteria. The larvae lived some 40 or 50 days, eventually reaching the fourth larval instar, but all died without pupating.

Experiment XIII.

A number of further trials were made, using insect broth and manure water. Eggs were transferred to 12 tubes of the former and 11 of the latter; in a few of these tubes sterility was maintained and some were used in Experiment XX.

In the majority of cases the eggs hatched in consequence of the development of moulds, or showed a feeble slow-growing bacillus. The results in the sterile tubes showed that very slow or no progress took place in them. The larvae which succeeded in growing finally died before their full size was attained and there was a suspicion in these cases of some feebly growing bacillus which had escaped the sub-culture test. In the more numerous instances where the presence of the feeble infection was proved the larvae seldom completed their development.

Experiment XIV.

Use of killed culture of B. coli.

Eggs placed in tubes containing broth cultures of *B. coli*, killed by heat, failed to hatch within a period of 5 days. A larva was transferred from a sterile tube in another experiment. This larva lived for a period of over a month, but failed to make any progress. The tube was finally inoculated from a living *B. coli* culture

when the larva died. This result has followed in a number of instances when the sterile conditions in which larvae have been living for a long period are broken down. In some instances no doubt this has been due to the violent character of the growth, with which two or three small larvae are quite unable to cope, but, apart from this, it would seem that some adjustment to the sterile conditions has occurred which cannot easily be reversed. In contrast to this, is the fact that larvae which are only starved by keeping them short of food in an unsterilized condition, are quite able to take advantage of an influx of bacteria, rapid development following immediately.

Experiment XV.

Development of larvae on a diet of Staphylococcus aureus.

(Note also tube No. 7, Experiment IX.)

Eggs sterilized by Method *b* were transferred by pipette into two tubes of peptone broth. One tube was inoculated from a *B. coli* culture, the other from one of *S. aureus.*

Growth of the bacteria followed immediately in the *aureus* tube and the eggs hatched within a few hours of inoculation.

In the *B. coli* tube the inoculation failed; only 1 egg hatched, and the larvae died within 3 days. On the 4th day the tube was again inoculated from the same culture of *B. coli*; by the following morning it was observed that the second inoculation had been effective and that the eggs had hatched.

The larvae in both tubes grew rapidly and produced adults within 8 to 15 days.

Experiment XVI.

Question of the necessity of solid particles with the food, also note respecting the effect of past bacterial action on the eggs.

It was suggested by one of our colleagues that the mosquito larvae might be unable to obtain nourishment in a pure fluid, particulate matter being essential to their digestive processes. We therefore tried the effect of adding animal charcoal to some tubes and not to others. Eggs that had been kept moist from laying were used and sterilized by Method *b*. The theory here was to allow of bacterial action on the outer surface of the eggs up to the moment of their sterilization. Apparently, on the showing of this one test, the theory has some basis as the result showed more eggs hatching under sterile conditions than usual.

The eggs were transferred to tubes of peptone water, peptone broth, pure beef broth, 2 % and 3 % solutions of an autolyzed extract of brewers' yeast in distilled water. To the yeast extract tubes a small quantity of animal charcoal was added prior to their sterilization.

Eggs hatched in some of the tubes within an hour and many more within two hours; such rapidity suggests past rather than present bacterial action, as even if the tubes were infected at the placing of the eggs in them, there was little time for growth, whereas all the experiments suggest that it is the vigorous bacterial action of a maximum infection which counts in causing hatching.

After 60 hours the following had emerged:

Peptone water	2 out of 16.	Sterile.
	0 „ 6.	„
Beef broth	all „ 18.	Infected.
	4 „ 11.	Sterile.
Peptone broth	1 „ 17.	Sterile; larva dead.
	7 „ 17	„
2 % solution yeast extract ...	several[1].	„
	„	„
3 % solution yeast extract ...	4 tubes, a few in each tube.	

It was noted that all the larvae in the yeast extract tubes containing charcoal were making progress, the dark line of their guts showing that the charcoal was being swallowed.

After 84 hours:

Peptone water	3 larvae from 16 eggs.	Sterile; no progress.
	0 „ 6 „	
Beef broth	all „ 18 „	Rapid progress of larvae; gas production by bacterial growth.
	6 „ 11 „	Very slight progress of larvae; still sterile.
Peptone broth	1 larva (dead) 17 „	Sterile.
	9 larvae from 17 „	Very slight progress of larvae; still sterile.
2 % solution yeast extract ...	4 larvae from about 15.	3 passed first moult.
	4 „ „ 15.	2 „ „
3 % solution yeast extract ...	1 „ „ 15.	Has passed first moult.
	4 „ „ 15.	Have „ „
	3 „ „ 15.	1 has passed first moult.
	5 „ „ 15.	3 have passed first moult.

With one exception the broth and peptone tubes passed out of the experiment owing to the larvae dying after a protracted period of minimal progress, or owing to sterility breaking down in the tubes, followed by the hatching of the dormant eggs and rapid development of the larvae.

Beef broth. In one tube of beef broth sterility was maintained; on the 41*st day* there were 4 living larvae in the third instar; on the 76*th day* tests showed that the tube was still sterile, some of the larvae having advanced to the fourth instar. On the 90*th day* 2 adults were reared; a small quantity of broth from the tube, together with one of the adult mosquitoes, was introduced into a tube of peptone broth which remained sterile. The original tube was infected with *B. coli* to see if any eggs were lying dormant; none hatched, so a few unsterilized eggs were added to test the nutritive properties of the broth after infection. Larvae emerged from the eggs the same day, by the 8th day nearly all had passed the third moult.

Yeast extract solution. Eggs were transferred after sterilization to a few tubes of 3 % solution of an autolyzed extract of brewers' yeast; no charcoal being added,

[1] The presence of animal charcoal in the tubes rendered an exact count impossible.

larvae hatched in some of these tubes, but made still slower progress than did those with charcoal, behaving as did the larvae in sterile peptone water or peptone broth, generally dying in their first instar and but very exceptionally surviving to the third.

2 % *solution of yeast extract with charcoal added.* After 50 days in one tube (No. 30) there were several larvae in the third instar and at least one in the fourth; 1 had pupated but the pupa died. All the larvae were lank and slender and had failed to develop the fat bodies which, towards the close of the larval life, become rather obvious owing to the opaque white appearance that they give to the larvae.

The second tube (No. 31) contained 4 larvae in their fourth instar, all deficient in fat deposits.

One tube (No. 32) contained a single weakly looking fourth instar larva; the emainder were dead.

Another (No. 33) contained 4 fourth instar larvae, all deficient in fat.

In tube No. 34 none of the larvae had got past the third skin. By the 56th day the larvae in tubes 31 and 32 were dead.

One larva was living in tube 30 on the 76th day, but died before the 95th day. In tube No. 33 two larvae lived until the 76th day and one until the 123rd day, when it died. In tube No. 34 two larvae lived until the 76th day; between the 76th and 95th days several larvae hatched from dormant eggs in this tube. Tests showed that sterility was still maintained; by the 107th day all the recently emerged larvae were dead except one, and this had passed the first moult; two died between the 107th and 123rd day. Between the 123rd and 143rd day moulds developed in the tube and the last surviving larva died.

Experiment XVII.

A further series of tubes containing a 3 % solution of autolyzed extract of brewers' yeast in distilled water, some with and others without charcoal, were prepared.

23 *days from start.*

No. 51,	sterile,	larvae.	Slender in second or third instar.
No. 52	,,	,,	Slender in third or fourth instar.
No. 53	,,	,,	Great disparity of growth in all instars from first to fourth skin; all slender.
No. 54	,,	,,	Slender in second or third instar.
No. 55	,,	,,	Great disparity of growth in all instars from first to fourth skin; all slender.

Tubes, *with charcoal*, 56 and 57.

No. 56, a mould grew, the larvae had grown steadily and were in fourth instar.

No. 57, sterile larvae show slow but healthy growth, are rather more robust than those in the tubes without charcoal, but are only in second and third skin.

40 *days from start.*

Nos. 51 to 55 *without charcoal.* No appreciable progress in the last 17 days.

Nos. 56 and 57 *with charcoal.*

No. 56, larvae have all died without pupating.

No. 57, no appreciable progress.

56 *days from start.*

Nos. 51 to 55 *without charcoal.*

No. 51, sterile. Most of the larvae in their third instar; one or two possibly dwarfed in fourth instar.

No. 52, sterile. Only 2 larvae living, 1 in third instar, 1 in fourth instar.

No. 53, a mould present; 1 ♀ reared, larvae in second, third and fourth instars, still living.

No. 54, sterile. All in third instar.

No. 55, sterile. 2 larvae in fourth instar; 1 pupa.

With charcoal.

No. 57, all the larvae now in third instar.

78 *days from start.*

Without charcoal.

No. 51, apparently sterile; little if any change.

No. 52, „ „ „ „

No. 53, mould present; most are in fourth instar.

No. 54, apparently sterile; little if any change.

No. 55, apparently sterile; 1 adult, 1 third or dwarfed fourth instar larva; 1 larva dead.

With charcoal.

No. 57, sterile; slight if any change.

90 *days from start.*

Without charcoal.

No. 51, sterile; no noticeable change.

No. 52, „ „ „

No. 53, mould has increased in size; only 1 larva living

No. 54, sterile; no noticeable change.

No. 55, „ „ „

With charcoal.

No. 57, sterile; slight progress.

106 *days from start.*

Without charcoal.

No. 51, sterile; no apparent change.

No. 52, sterile; dormant eggs have hatched; there are now 5 living larvae.

No. 53, all dead, the last surviving larva died on or about the 95th day.

No. 54, sterile; no noticeable change.

No. 55, „ „ „

With charcoal.

No. 57, sterile; only 1 larva now living.

126 *days from start.*

Without charcoal.

No. 51, sterile; little if any change; 3 larvae still living.

No. 52, sterile; 4 larvae living; those recently hatched have made slight progress.

No. 54, sterile; 2 larvae living; little if any change.

No. 55, sterile; 2 larvae living; 1 in fourth and 1 in second instar.

With charcoal.

No. 57, the last larva is now dead.

Evaporation had so reduced the quantity of fluid that boiled distilled water was added to tubes Nos. 51, 52, 54 and 55. Following the addition of the water a few eggs that had lain dormant in tubes Nos. 51, 54 and 55 hatched.

Nine days later, 135 *days from start.*

No. 51, it was found that all the larvae were dead.

No. 52, 1 of the 4 larvae died.

No. 54, only 1 of the 2 original larvae living; those that hatched out recently are dead.

No. 55, the recently hatched larvae have disappeared, possibly swallowed by the larger ones.

Tube No. 54 inoculated with a yeast (*S. cerevisiae*).

142 *days from start.*

No. 52, all the old larvae are dead, the last two died with their jaws entangled in a small tuft of hair. The three survivors are growing steadily. (Sterile.)

No. 54, this tube was inoculated with a yeast (*S. cerevisiae*); the surviving larva after one week looks large and stout in comparison with its dead companions.

No. 55, the surviving old larva is still active, two of those which hatched after the addition of fresh water are living and are now in second skin. (Sterile.)

152 *days from start.*

No. 52, moulds grew in the tube; the three last larvae died.

No. 54, the surviving larva pupated.

No. 55, the old larva is dead and also one of the recently hatched ones.

Tube No. 55 was inoculated with (*S. cerevisiae*).

168 *days from start.*

No. 54, the pupa died.

No. 55, an adult ♀ was reared from the surviving larva in this tube.

Experiment XVIII.

Killed B. coli *culture and manipulated milk.*

A tube of distilled water, to which washed *B. coli* had been added, was sterilized by heat, and a sterile larva introduced that had hatched out in peptone broth. After 13 days during which the larva made no progress, 1 c.c. of sterilized milk, the colloid particles of which were increased in size but not precipitated by a manipulation of its acidity, was added. No progress occurred within 3 days; the tube was inoculated from a tube of broth in which a certain species of bacterium had developed. (This culture was used because its speed of growth under the conditions of the experiment had allowed the larva in the tube to develop satisfactorily.) The larva at once commenced to grow, and pupated within 20 days, an adult emerging in due course.

Experiment XIX.

A number of tubes were prepared and sterilized containing appropriate quantities of the following in distilled water; sterile filtrate from a culture of *B. coli* with white of egg, treated so as to form a fine precipitate; filtrate of *B. coli* with milk in which the casein particles had been increased in size; and *B. coli* filtrate without any addition.

Larvae were taken from a sterile tube of peptone water in which they had hatched 16 days previously; they were still in the first instar, having made barely perceptible progress during 16 days. Slight progress followed over a protracted period in the tubes (containing white of egg and milk) which remained sterile, but in all cases it came to a standstill when the larvae were still small, in the second or third skin, after which they pined away and died. In one or two instances sterility broke down and was generally followed by a sudden spurt of growth. In most cases, however, the growth of bacteria proved over-violent and killed the insects ere they had time to develop.

Experiment XX.

The treated milk was also added to tubes of yeast extract solution, peptone water and manure water, in which eggs had hatched and the larvae were unable to develop owing to the sterile conditions. In no case did the addition of the milk produce any change in the scarcely perceptible progress that the larvae made. Sterile filtrate of *B. coli* in the proportion of about 1 in 10 was added to these tubes after the lapse of 10 or 12 days, and also to sterile tubes of insect broth and 3 % yeast extract solution. During 5 days no change resulted from the addition of the filtrate, the scarcely perceptible growth that was taking place in some only of the tubes was not appreciably quickened, in other cases when the larvae had already arrived at the marking time phase no progress was induced.

The following notes refer to some of the tubes mentioned above.

Insect broth. A larva that had hatched under sterile conditions (see Experiment XIII) lived in the tube for 34 days when it was still in the first skin; 1 in 7 of *B. coli* filtrate was added. No change took place in 5 days; then the tube was inoculated with *B. coli*; eggs which had been lying dormant in the tube for 39 days hatched as a result. Rapid growth of the larvae followed, but it is not possible to say definitely if the larva which had been marking time for so long was one of those which developed to adult stage within 10 days; 4 had pupated and 3 ♂♂ and 1 ♀ emerged in due course.

Peptone water. The two or three larvae which hatched from the eggs pipetted into the tube made no progress during 18 days; the treated milk was added—the larvae made no progress and died out within three weeks. Eggs had been lying dormant in this tube for 39 days. The tube was inoculated with *B. coli* and these eggs hatched within a few hours. The larvae commenced to make rapid progress, but their history was not followed up.

Yeast extract in distilled water. Three or four larvae hatched from the eggs placed in this tube. They made no perceptible progress during a week; treated milk was added to the tube. After 20 days two of the larvae had passed the first moult; 76 days after the addition of the milk the larvae were still in the second or

third instar, and one had failed to complete its moult satisfactorily. Three larvae were still living on the 88th day after the addition of the milk; two were living in the same stage after 104 days and one after 124 days. Sterile water was then added to the tube to replace evaporation and the tube was inoculated from a *B. coli* culture; the violence of the growth, however, killed the larvae. (Eggs were added to the tube; they hatched almost immediately and the larvae were killed off within a day, showing that the death of the larvae which had lingered on for so long a time was not in all probability connected with their age.)

Various egg hatching and larva feeding tests.

Experiment XXI.

Sterile tubes prepared of filtrate from a *B. coli* culture; *B. coli* filtrate and precipitated white of egg; *B. coli* filtrate and milk in which the casein particles had been enlarged; autolyzed yeast extract in distilled water; peptone water; and distilled water.

Eggs sterilized by Method *b* were pipetted into each tube.

No. of eggs hatched in	Tubes	2½ hours	20 hours	48 hours	96 hours
Filtrate of *B. coli* culture	1	2	3	All hatched*	All* larvae growing rapidly
	1	1	1	All hatched*	All* larvae growing rapidly
	1	0	1	All hatched*	All* larvae growing rapidly
	1	0	0	1	All* larvae growing rapidly
	1	0	0	0	2, hatching became general and a mould appeared
	1	0	0*	Used for special test, see Experiment IV, page 503.	
Filtrate of *B. coli* and precipitated white of egg	1	0	1	All hatched*	Larvae growing rapidly
	1	0	0	All hatched*	Larvae growing rapidly
Filtrate of *B. coli* and treated milk	1	0	1	All hatched*	Larvae growing rapidly
Autolyzed extract of brewers' yeast, in distilled water	1	0	0	2	No change
	1	0	0	0	
	1	0	0	0	
Peptone water	1	1	1	1	No change
	1	0	0	0	1
Distilled water	1	1	5	6	10
	1	1	3	3	5
	1	0	2	Used in a special test; see Experiment IV, page 503.	
	1	0	2		
	1	0	2		
	1	0	1		

* Tested for sterility—all proved to be infected.

After 120 *hours.* The larvae in all the starred (infected) tubes continued to grow rapidly: in the filtrate tube in which a mould appeared, all the eggs hatched but the larvae made no progress.

Films from the filtrate tubes in which sterility broke down stained by Gram's method showed a mixed infection of bacteria other than *B. coli.* One tube showed sporing forms. The results were consistent with the explanation that the tubes were infected by organisms on the eggs, some of which had escaped the sterilizing process.

Of the two peptone water tubes all the eggs in one tube hatched; a mould was noticed developing in the tube. The larvae made little if any progress during a period of 20 days, the mould gradually increasing at the surface of the fluid. The larvae were all dead within a month of hatching. 1 c.c. of the sterile filtrate of *B. coli* was added to the remaining peptone water tube and the two tubes of distilled water. Within 4 days all the eggs hatched in the peptone water tube, but it was evident that sterility had broken down. The larvae made rapid progress, but failed in their race with the bacteria—the fluid in the tube becoming very foul—and the larvae died.

Sterility also broke down in the two tubes of distilled water, to which the filtrate was added. It is quite possible that it had broken down at the time when the eggs began to hatch, but as the tube was not tested the fact was not apparent until the filtrate was added. The larvae made rapid progress at first, but it soon appeared that they had exhausted the food supply as, although they continued healthy, no progress was made. The larvae gradually died off—a few survivors lingered on until the 88th day; they were then small and undersized in the third or fourth instar. Moulds were developing in both tubes and the death of the larvae followed in a few days. Concerning the filtrate tubes to which precipitated white of egg and treated milk were added nothing of interest remains to be said; all were infected with a species of bacterium that required cool conditions—60° to 65° F.—for its favourable development. The larvae grew rapidly at first but slowed down after a few days; after the 8th day they were still in the third instar. Those in the tube to which milk was added attained the fourth instar by the 12th day and then died. The larvae in one of the white of egg and filtrate tubes developed slowly and 2 adults were reared within a month, but a mould had developed in the tube, and the remaining larva died. In the other tube a mould also developed and all died as larvae.

3 % *Yeast extract solution.* Nos. 106 and 107. No larvae hatched within 96 hours, but one was out in each tube within 120 hours; these larvae died within 2 or 3 days. In one tube no more eggs hatched within 10 days; in the other a few larvae emerged and died. On the 12th day both tubes were inoculated from a culture of a yeast; the eggs hatched during the night, in one tube they all died, but in the other a few of the larvae made rapid progress. Three ♂ specimens were reared within 18 days of the inoculation and three ♀♀ followed a week or so later.

2 % *Yeast extract solution with charcoal.* One tube; within 24 hours 6 or 7 larvae had hatched, the larvae making slight if any progress within 3 days of hatching; within the next few days moulds commenced to grow. By the 7th day after hatching a few larvae had passed their first moult. Some of the larvae attained to the fourth instar within 20 days of hatching, but the growth of moulds had made

considerable progress by this time, and the larvae died within 2 or 3 days. *Infection by bacteria in addition to the moulds was probable.*

Experiment XXII.

Planned to contrast the effect, if any, on hatching of *B. coli* filtrate, beef broth and distilled water. It failed owing to the number of tubes in which sterility broke down. The experiment, however, affords evidence relative to the effect of the growth of moulds and the ability of the larvae to develop on a diet of living yeast cells.

To each of 9 tubes containing 6 c.c. of sterile distilled water, was added 1 c.c. of filtrate from a culture of *B. coli.*

To each of 6 tubes was added 1 c.c. of plain beef broth.

Eight tubes contained the distilled water only.

Eggs were sterilized by Method *c* and from 12 to 20 pipetted into each tube.

After 24 hours.

Filtrate and water. 7 of the tubes have at least 20 eggs; 2 have only 12 in them. In all the tubes there are one or more larvae swimming. In most of the tubes there are 5 or 6 larvae; in one 8 or 9, and in one a dozen.

Beef broth. Four of the tubes had 12 to 18 eggs; two had at least 20 eggs in them. The hatchings were: 2 : 1 : 2 : 1 : 8 : 2.

Distilled water. Six of the tubes had from 12 to 20 eggs; two had 20. The hatchings were: 1 : 1 : 0 : 0 : 0 : 2 : 0 : 0.

After 4 days.

Filtrate and water. In one tube (No. 9) there was a bacterial growth; the water was cloudy; all the eggs had hatched and the larvae showed vigorous progress. In all the remaining tubes, though hatching was general, larval progress was not evident; there was no bacterial growth, but moulds had commenced to grow in the tubes.

Beef broth and water. In two tubes (2 and 6) bacterial growth started; in two (1 and 5) moulds grew, while two (3 and 4) remained sterile. Hatching was general, but not complete in those tubes which were not infected by bacteria or moulds; larval growth was rapid only in those containing bacteria.

Distilled water. In two of the tubes two or three eggs hatched; in three one hatched, while in three no larvae emerged.

Subsequent history.

Filtrate and water. Nine tubes numbered 1 to 9.

No. 1 had a double infection of bacteria and moulds; one undersized ♂ bred after 35 or 40 days, remainder died.

No. 2, white surface moulds gradually increased, and the larvae gradually died off; 1 lived 60 days, and died in the third instar.

No. 3, dark surface moulds grew gradually; 1 larva lived 53 days but died in the third instar.

No. 4, grey surface moulds grew gradually; none of the larvae passed the second moult—all died within 50 days.

No. 5, the mould in this tube was more vigorous; it formed flocculent tufts just beneath the surface. The last larva to die was in its third instar. None lived beyond 50 days.

No. 6, white surface moulds grew gradually; 3 larvae were living on the 53rd day; the last survivor died in the third instar within 60 days.

No. 7, in addition to floating moulds, this tube had a heavy bacterial growth in it. 2 fourth instar and 1 second instar larvae were living on the 69th day, but all died without reaching pupal stage.

No. 8, small globular moulds developed at the bottom of the tube; all the larvae died in the first or second instar early in the course of the experiment.

No. 9, this tube had an obvious infection, a surface scum formed, but there was no trace of masses of mould, as in the other tubes; 5 larvae lingered in the second and third instars until the 53rd day, but all died out by the 69th.

Beef broth and water.

No. 1, a white surface mould; all larvae died in first and second skin.

No. 2, a weak, double bacterial infection; larval progress slight; several larvae in second and third skin were living on 53rd day; by the 106th day 1 larva was still living and a sub-culture in broth gave no result, the infection being presumably too slight. 1 c.c. of broth was added to the tube and subsequently ·5 c.c. from the tube was added to sub-culture broth tube. Result: infected. A larva hatched from a dormant egg within two hours of the addition of broth, presumably as the result of increased bacterial growth consequent upon the addition of fresh nutritive material.

No. 3, this tube remained sterile to all the tests applied; 1 larva reached second and 1 the third skin by the 53rd day; both died subsequently.

No. 4, this tube remained sterile; 5 larvae were still living in the first instar on the 21st day; on the 28th day the larvae showed little if any progress. A mass of living yeast cells (*S. cerevisiae*), lifted from a wort agar slope, was added to the tube; several dormant eggs hatched within a few minutes. The larvae all grew rapidly and cleared the water, which became cloudy when the yeast cells were introduced, within 5 days. A fresh loopful of the yeast cells was added. In 2 days' time the water was again clear, and a fresh loopful was given. On the 13th day most of the larvae were in their fourth skin and one had pupated. Another loopful of the yeast cells was given. A week later a male was reared and a loopful of yeast cells added. A second adult, a ♀, was reared on the 25th day after infection. A further loopful of the yeast cells was given; by the 32nd day 3 adults had been reared. There were 2 pupae and 4 fully developed larvae; further yeast cells were given. In all 8 adults were reared in 40 days. The period taken in this instance was due to the lapse of time between the meals and the small allowance of food given at any one time; had a larger mass been given, not above one-third of the time would have been taken.

No. 5, several very small moulds grew at the bottom of the tube. The larvae made slight progress; the moulds did not develop, and apparently died out. The larvae made very slight progress, but by the 40th day after hatching none had passed the second instar. Some of the larvae died, others made slow progress. After 100 days there were 2 living, 1 in the third and 1 in the fourth instar. Tested and found sterile on the 100th day.

No. 6, this tube developed a vigorous bacterial growth; the larvae made rapid progress, but all died in the second or third skin.

Distilled water. Of the eight tubes to which eggs were added only one showed infection by the tests used; on the 20th day of the experiment the position was as follows:

No. 1, 2 eggs hatched, 1 larva dead, 1 living, both in first skin.

No. 2, none hatched.

No. 3, 3 hatched, 1 larva dead, 2 living, all in first skin.

No. 4, 6 hatched, all dead in first skin.

No. 5, none hatched.

No. 6, 5 hatched, 4 dead, 1 living, all in first skin.

No. 7, many hatched, probably all, larvae all dead but one; none past first skin, infected by mould.

No. 8, 1 hatched; larva living in its first skin.

All the living larvae died within a week or two; the tubes containing dormant eggs were used for the under-noted experiment.

On the 28th day of experiment a loopful of living yeast cells (*S. cerevisiae*) was added to *tube No.* 1. Eggs which had been lying dormant for 28 days hatched within 5 minutes; rapid growth ensued and from time to time a fresh loopful of the yeast cells was added. 5 adults were reared and one specimen died in pupal stage. The time taken was from 30 to 50 days; the quantity of food given at five-day or weekly intervals—was a small mass of cells scraped from a wort agar slope with a platinum loop—only allowing of development by fits and starts.

Tubes Nos. 2, 3, 4, 5, 6 and 8 were infected by adding ·5 c.c. of a yeast culture; larvae hatched from dormant eggs in most of the tubes within 5 minutes; others followed rapidly.

All the larvae made rapid progress except those in tube No. 5; here moulds started to grow rapidly. Within a week there was only one larva living, and this one also died within a day or two.

A small quantity, usually about ·2 or ·3 c.c. of the yeast culture, was added at intervals of several days or a week; the point it was wished to establish being not the speed with which the larvae could develop on a diet of yeast cells, but the fact that they marked time during the intervals and spurted when the food was given. In spite of the rather crowded condition of the larvae in 6 or 7 c.c. of fluid, they remained healthy, except in tube No. 4 where moulds developed. In this tube all the larvae died. The result showed as anticipated the entire dependence of the larvae on the added yeast culture, their speed of progress being conditioned by the length of the periods between each addition to the tubes. In tube No. 2, 6 adults were reared, in tube No. 3, 6, and 6 individuals in tube No. 6; while in tube No. 8 which contained more larvae to start with 9 adults were reared.

Experiment XXIII.

The increasing proportions of tubes in which sterilization broke down either from sporing species of bacteria or moulds, suggested that the contamination of the eggs laid in the breeding-pan was very gross. Steps were therefore taken to obtain eggs laid under more cleanly conditions and less likely to be heavily infected with sporing bacteria and moulds (the method adopted is described on pp. 488–9).

Eggs were used that had had time to incubate, but had not dried; these were well washed in three changes of clean tap water, put on to clean filter paper and then dried; they were subsequently sterilized by washing in weak ·5% lysol, then in 2%, then in 1%, and then in sterilized distilled water; 9 or 10 eggs were transferred by Pasteur pipette to the following tubes:

Distilled water, Nos. 1 to 6.

Distilled water with 1 c.c. of sterile *B. coli* filtrate (=1 in 6), Nos. 7 to 12.

Beef broth (without peptone or salt), Nos. 13 to 18.

Beef broth with 1 c.c. of sterile *B. coli* filtrate (= 1 in 6), Nos. 19 to 24.

Media	No. of tube	Hatching after 24 hours	After 5 days	Remarks after 5 days
Distilled water	1	0	0	The single larva which emerged did not grow. In one or two other tubes eggs uncapped, but the larvae died without quitting the shell.
	2	0	0	
	3	1	1	
	4	0	0	
	5	0	0	
	6	0	0	
Distilled water and filtrate of *B. coli* (= D.W. 6 c.c., F. 1 c.c.)	7	0	0	Sterility broke down after 24 hours in Nos. 9, 10 and 11, and the larvae which emerged made rapid progress.
	8	0	0	
	9	2	All*	
	10	0	All*	
	11	3	All*	
	12	0	3	
Beef broth without salt or peptone	13	2	All*	Heavy growth of bacteria, larvae already in fourth instar.
	14	1	1	Larva makes no progress.
	15	0	1	Larva makes scarcely any progress.
	16	0	1	Larva makes no progress.
	17	0	0	
	18	1	1	Larva makes slight progress.
Beef broth with filtrate of *B. coli* added (=B.B. 6 c.c., F. 1 c.c.)	19	0	0	
	20	0	2	Larvae show no progress.
	21	0	1	Larva shows no progress.
	22	0	7*	Heavy bacterial growth, larvae are already in fourth instar; remaining eggs failed to hatch.
	23	0	0	
	24	1	1	Larva shows no progress.

On the 6th day tube No. 15 was inoculated from No. 13; all the eggs had hatched by the following morning.

After the 7th day.

Distilled water and Filtrate. There was a distinct difference as regards the progress of the larvae in the infected tubes 9, 10 and 11; while the larvae in 9 and 11 were on a parity as to size, those in 10 were slightly but definitely smaller. Sub-

* Infected tubes.

cultures from these tubes show that, while the bacteria infecting 9 and 11 are closely allied, probably the same species, the infection of 10 differs in that it shows a much less vigorously growing species. The interesting point is that, although the difference in larval progress is slight, the divergence between the vigour of bacterial growth is extreme.

After the 13th day.

Distilled water. Tubes 1 to 6. The only larva which emerged has made no progress and is now very feeble.

Distilled water and Filtrate. The larvae in tubes 9, 10 and 11 are all of full size, but none of them have as yet pupated. In No. 12 three more eggs have hatched, none of the 6 larvae have as yet passed their first moult.

Beef broth. In tube No. 13 all the larvae have been killed by the vigour of the bacterial growth. In No. 14, 3 more eggs have hatched. In No. 15 the larvae are now either just approaching or past the second moult. This tube was passed on to a friend and kept at a lower temperature than the others—60° F. instead of 75° F. In No. 16 another egg has hatched, neither of the two has made any progress and one has died. In No. 17 one egg has hatched. In No. 18, 5 more eggs have hatched, but only the larva which emerged 12 days ago has made any progress and this one has not yet moulted.

Beef broth and Filtrate. No. 20, one of the two larvae is dead; their progress since hatching is only just perceptible.

No. 21, the larva has died without moulting.

No. 22 (infected), 7 adults have been bred, 6 ♂♂ and 1 ♀; 1 living pupa remains.

No. 23, 1 egg has hatched.

No. 24, the larva has died without moulting.

After the 21st day.

Distilled water. 1 to 6; the larva which emerged in tube 3 died in its first instar.

Distilled water and Filtrate. In tubes 9 and 10 the larvae are marking time in their fourth instar. In No. 11, 2 ♂♂ have been bred, the remaining larvae, of full length, are marking time. In No. 12 the 6 larvae make scarcely perceptible progress.

Beef broth. In tubes Nos. 14, 16, 17 and 18 the larvae are making very slow progress; a few are approaching, and one or two have passed their first moult. In tube No. 15, inoculated from No. 13 and passed on to a friend, the larvae made comparatively slow progress, presumably owing to the low temperature at which the tube was kept; apparently the vigour of the bacterial growth was the cause of their destruction.

Beef broth and Filtrate. In tube No. 19 one egg uncapped, but the larva was either already dead or died immediately afterwards, as it failed to get clear of the eggshell.

No. 20, 2 more eggs have hatched. The survivor of the first two larvae is now in its second instar.

No. 22 (infected), another adult was reared (8 in all).

No. 23, the larva from the only egg which hatched is now in its second instar.

No. 24, another egg has hatched; the larva is now in its second instar.

After 28 days.

Distilled water. No change; eggs still dormant.

Distilled water and Filtrate. Nos. 9 and 10, the larvae still marking time.

No. 11, another adult reared; some of the larvae have died, only 1 is now living.

No. 12, only 4 of the 6 larvae are living; all the larvae are still in their first instar.

Tubes Nos. 7 and 8 were used in a demonstration at a meeting of the Entomological Society of London. To No. 7 was added a small mass of yeast cells from a wort agar culture of *S. cerevisiae* and to No. 8 a small mass from a culture of *B. coli*. The eggs in No. 7 hatched within a few minutes, those in No. 8 during the following night.

Beef broth. No. 14, 1 larva in first and 3 in the second instar.

No. 16, 3 larvae in first and 2 in second instar.

No. 17, 4 larvae in first and 1 in the second instar.

No. 18, 1 larva has died; 4 are still in their first and 1 is in its second instar.

Beef broth and Filtrate. No. 19, no change.

No. 20, another egg has hatched; 3 larvae are in their first and 1 in its third instar.

No. 21, no change.

No. 23, another egg has hatched; the first larva is still in its second instar.

No. 24, 2 more eggs have hatched; the older larva is now in its third instar.

After the 33rd day.

Distilled water. No change.

Distilled water and Filtrate. Nos. 7 and 8, there is a marked difference in the growth of the larvae in these two tubes. Approximately the same bulk of *B. coli* was put into No. 8 as there was of yeast cells into No. 7. The larvae in No. 7 have only reached the second and third instars while those in No. 8 are all either in the third or fourth instar. This difference can hardly be owing to a minute difference in mass of the bacteria or yeasts which were added; probably it is due to the medium (distilled water with a little filtrate of a broth culture of *B. coli* added) being better adapted for the multiplication of bacteria than the yeasts. The fourth instar larvae in tubes 9 and 10 and the remaining larva in tube 11 are all marking time, having presumably exhausted all the nutriment without fully developing their fat bodies. In tube 12 all the larvae, save one, died in their first or second instars; the survivor, still in its first instar, is only capable of feeble movement.

Beef broth. No. 14, 1 larva is dead.

No. 16, no noticeable change.

No. 17, two larvae have died, the survivors are 1 in second and 1 in third instar.

No. 18, no change.

Beef broth and Filtrate. Nos. 19 and 20, no change.

No. 21, the larva which was in its second instar is dead.

No. 23, no change.

No. 24, only 1 larva in its first instar is now living.

After 49 *days.*

Distilled water. No change.

Distilled water and Filtrate. No. 7, these larvae are now in their third and fourth instars.

No. 8, 4 adults have been reared (within 21 days); there still remain 1 pupa and 1 full-grown larva in the tube.

No. 9, all the larvae but one are dead.

No. 10, 1 adult has been reared; there are still 4 full-grown larvae in the tube.

No. 12, the only larva is dead.

Beef broth. No. 14, no change.

No. 16, 1 has died; the 3 survivors have made no appreciable progress.

No. 17, the larva in its second instar has died, but 2 more eggs have hatched; the remaining larva is still in its third instar.

No. 18, 2 of the larvae in first instar have died.

Beef broth and Filtrate. No. 19, no change.

No. 20, 1 of the larvae has died.

Nos. 21, 23 and 24, no change.

After 50 *days.*

Beef broth and Filtrate. Tube No. 19 was inoculated from a culture of *B. coli* on the 50th day; 8 or 9 eggs hatched within a few hours.

After 60 *days.*

Beef broth and Filtrate. About ·5 c.c. of a broth culture of *B. coli* was added to tube No. 21 on the 60th day. A number of dormant eggs hatched within 10 to 15 minutes.

After 69 *days.*

Distilled water. No change.

Distilled water and Filtrate. No. 7, 1 adult reared; 1 died in pupal stage; 3 fourth instar larvae still living. A slow growing surface mould is present.

No. 8, 2 more adults reared (6 in all).

Nos. 9, 10 and 12, no change.

Beef broth. No. 14, another larva has died; the 2 survivors appear very feeble.

No. 16, all the larvae are dead.

No. 17, 1 of the 3 larvae is dead.

No. 18, 1 of the 2 larvae is dead.

Beef broth and Filtrate. No. 19, 5 adults reared, 1 larva died when full grown.

(Note these eggs were dormant for 50 days and the adults were reared within 20 days of inoculation of the tube with *B. coli.*)

No. 20, 1 larva has died.

No. 21, the larvae which hatched in this tube after its inoculation with *B. coli* were full grown within 7 days.

No. 23, the second instar larva is dead; another egg has hatched.

No. 24, no change.

After 78 days.

Distilled water. No. 7, a second adult reared; 1 larva living, the others died in fourth instar.

No. 9, the last larva has died.

No. 10, a second adult reared; 3 living larvae remain.

No. 12, no change.

Beef broth. No. 14, both the larvae are dead.

No. 17, both the larvae are dead.

No. 18, the larva is dead.

Beef broth and Filtrate. No. 20, no change.

No. 21, 7 adults reared within 16 days of the hatching of the eggs.

No. 23, no change.

No. 24, the third instar larva is dead; only 1 of the younger larvae is living, it is still in its first instar.

After 87 days.

Distilled water. No change.

Distilled water and Filtrate. No. 7, 1 larva marking time; moulds are growing out of the mouths of the dead larvae.

Nos. 10 and 12, no change.

Beef broth. No change.

Beef broth and Filtrate. No. 20, only 1 first instar larva is now living.

No. 21, the last larva has died.

Nos. 23 and 24, no change.

After 100 days.

Distilled water. Tubes 4, 5 and 6 were used for the following experiment in conjunction with Experiment XXIV, page 530.

To tube No. 4 about 3 % of a living culture of *B. coli* was added.
„ No. 5 „ 3 % of a sterile autolyzed extract of brewers' yeast.
„ No. 6 „ 3 % of a killed culture (autoclaved) of *B. coli.*

Result: No. 4, 1 hatched within 2 minutes; 2 more within 5 minutes; 2 more within 10 minutes.
No. 5, 1 hatched within 15—20 minutes.
No. 6, 1 „ „ „ „

After an hour and a half:
No. 4, 8 out of 10 eggs hatched.
No. 5, 1 „ 9 „ „
No. 6, 1 „ 11 „ „

A small quantity of living yeast cells from a wort agar culture was then transferred by platinum loop to tube No. 5 and the remaining 8 eggs hatched within 15 minutes.

About 3 % of a living broth culture of *B. coli* was added to No. 6, and 1 egg hatched within 3 minutes, another followed within 5 minutes and 4 more within 15 minutes = 6 out of 10.

After 110 *days.*

Distilled water. Nos. 1, 2 and 3, no change.

No. 4, larvae now in second instar.

No. 5, ,, ,, third and fourth instars.

No. 6, ,, ,, second and third instars.

The rapid growth of the larvae in tube No. 5 should be noticed in contrast to what happened with regard to tubes Nos. 7 and 8, distilled water and filtrate, after infection with yeasts and bacteria on the 28th day. Presumably this difference is due to the media in tube No. 7 having been unfavourable for the yeast.

Distilled water and Filtrate. No. 7, a further development of moulds; the larva is dead.

No. 10, all died as larvae with the exception of one which reached the pupal stage.

No. 12, an egg had hatched.

Beef broth. Nos. 14, 16, 17 and 18, all the larvae in these tubes are dead; possibly some living eggs remain dormant.

Beef broth and Filtrate. Nos. 20, 23 and 24, all the larvae are dead; possibly some living eggs are lying dormant.

After 134 *days.*

Distilled water. Nos. 1 and 3, small white moulds developed in the tubes.

Nos. 4, 5 and 6, want of nutriment checked development; a few larvae are living in their second and third instar but many are dead.

Distilled water and Filtrate. No. 10, another adult reared.

No. 12, another egg hatched; both the larvae which hatched after the 87th day and this one which hatched after the 110th day are in their first instar.

Beef broth. No larvae are living in these tubes, but it is possible that there may be dormant eggs.

Beef broth and Filtrate. In No. 23 an egg has hatched since the 110th day.

After 156 *days.*

Distilled water. Tube No. 2 was infected with *B. coli* without result; it was concluded that all the remaining eggs were dead.

Nos. 4, 5 and 6, 1 larva was living in No. 4 and 3 in No. 5; all were dead in No. 6.

Distilled water and Filtrate. No. 12, only 1 larva living.

Beef broth. No. 14, tested with *B. coli* for dormant eggs; none hatched.

No. 16, an egg had hatched since the 134th day.

Nos. 17 and 18, a little of a broth culture of *B. coli* was added; dormant eggs hatched in both tubes within 15 minutes.

Beef broth and Filtrate. The larva which hatched out of a dormant egg in No. 23 is dead.

An egg hatched in tube No. 20 after the 134th day.

After 203 *days.*

All the larvae are dead except the one in distilled water and filtrate No. 12; this is now in its fourth instar. One adult was reared in tube No. 5, distilled water.

The remaining tubes were infected with a yeast to see if any dormant eggs survived. Single eggs hatched in tubes No. 1 distilled water and No. 6 broth and filtrate. Further quantities of yeast cells were added to distilled water and filtrate tube No. 12 on the 204th day; the larva pupated on the 205th day and an adult ♂ was reared on the 208th day.

Experiment XXIV.

Planned to test further the effect of sterile cultures of bacteria and extracts of yeasts on dormant eggs.

Twenty-four tubes each containing 8 c.c. of distilled water were sterilized and a number of sterile eggs varying from 20 to 50 were introduced. After 5 days the tubes were examined, and a selection made of those in which only a few eggs had hatched.

The following were chosen:

No. 1,	about	50	eggs,	6	hatched;	all the larvae dead.
No. 2	,,	25	,,	none	,,	
No. 3	,,	40	,,	2	,,	all the larvae dead.
No. 4	,,	30	,,	1	,,	larva dead.
No. 5	,,	50	,,	4	,,	1 larva living.
No. 6	,,	45	,,	3	,,	all the larvae dead.
No. 7	,,	30	,,	3	,,	2 larvae living.
No. 8	,,	30	,,	3	,,	all the larvae dead.
No. 9	,,	25	,,	none	,,	
No. 10	,,	25	,,	2	,,	all the larvae dead.
No. 11	,,	40	,,	2	,,	,, ,, ,,
No. 12	,,	20	,,	1	,,	larva dead.
No. 13	,,	35	,,	5	,,	1 larva living.
No. 14	,,	30	,,	4	,,	2 larvae living.

Ten tubes were not used.

The cultures or extracts used were as follows:

Autolyzed extract of brewers' yeast, *B. coli*, *Staphylococcus aureus*, sterilized by autoclaving. *B. coli* and *S. aureus*, sterilized by steaming. *B. coli*, killed by chloroform and a sterile filtrate of *B. coli*. This last was several weeks old at the time of the experiment.

All the media had been kept for 48 hours and sub-cultured to test for sterility before use; ·5 c.c. = 6 % was added to each tube except in the case of the autolyzed yeast in which test ·2 c.c. = 2·5 % was added to one tube and ·5 c.c. = 6 % to the other.

Autoclaved:

	No. of Tube		
Autolyzed yeast extract...	No. 1, 2·5 % added	Hatching commenced within 5 minutes.
	No. 2, 6 % ,,	
B. coli	No. 3, 6 % ,,	Hatching commenced within 5 minutes.
	No. 4, 6 % ,,	
S. aureus	No. 5, 6 % ,,	No effect within 10 minutes.
	No. 6, 6 % ,,	

Steam heated:

	No. of Tube		
B. coli	No. 7, 6 %	added	Hatching commenced within 5 minutes.
	No. 8, 6 %	,,	
S. aureus	No. 9, 6 %	,,	No effect within 10 minutes.
	No. 10, 6 %	,,	

Killed with chloroform:

B. coli	No. 11, 6 %	,,	Hatching commenced within 10 minutes.
	No. 12, 6 %	,,	
Filtrate of *B. coli* ...	No. 13, 6 %	,,	No effect within 10 minutes.
	No. 14, 6 %	,,	

After half-an-hour.

Autoclaved cultures:

Autolyzed yeast extract... Nos. 1 and 2, all or a high percentage hatched.
B. coli ... ... Nos. 3 and 4, ,, ,, ,, ,,
S. aureus ... ... Nos. 5 and 6, ,, ,, ,, ,,

Steam heated:

B. coli ... ... Nos. 7 and 8, all or a high percentage hatched.
S. aureus ... ... No. 9, only one or two had hatched.
No. 10, all or a high percentage had hatched.

Killed by chloroform:

B. coli ... ... Nos. 11 and 12, a large number but certainly not all.
Filtrate of *B. coli*... ... Nos. 13 and 14, no effect.

After 18 *hours.*

Autoclaved:

Autolyzed yeast extract... Nos. 1 and 2, all hatched.
B. coli ... ... Nos. 3 and 4 ,,
S. aureus ... ... Nos. 5 and 6 ,,

Steam heated:

B. coli ... ... Nos. 7 and 8 ,,
S. aureus ... ... Nos. 9 and 10 ,,

Killed by chloroform:

B. coli ... ... Nos. 11 and 12 ,,

Filtrate:

B. coli ... ... Nos. 13 and 14, a good proportion but by no means all hatched.

It is to be noted that considerable numbers of eggs hatched out during this period in some of the remaining 10 tubes to which no addition had been made.

After 11 *days.*

Notes regarding the progress of the larvae.

Autoclaved:

Autolyzed yeast extract... No. 1, infected bacterial growth; larvae in second and third instar, 2 in fourth.
No. 2, sterile, larvae still in first instar; no progress.

		No. of Tube
B. coli	...	No. 3, infected, larvae still in second instar; growing.
		No. 4, sterile, larvae still in first instar; no progress.
S. aureus	...	No. 5, apparently sterile, larvae still in first instar; no progress.
		No. 6, apparently sterile, larvae still in first instar; but growing.
Steam heated:		
B. coli	...	No. 7, ? infected } Larvae growing in first and
		No. 8, ? infected } second instars.
S. aureus	...	No. 9, infected, larvae mostly in second instar, growing.
		No. 10, sterile, larvae still in first instar; no progress.
Killed by chloroform:		
B. coli	...	No. 11, infected, and a mould growing, larvae of varied growth, first to third instars.
		No. 12, sterile, larvae in first instar; no progress.
Filtrate:		
B. coli	...	No. 13, infected, all eggs hatched, larvae in second or third instars; growing.
		No. 14, sterile, a number still dormant; larvae in first instar; no progress.

Unfortunately time did not permit of sub-culture tests to clear up doubtful points as to infection of tubes.

The suggestion conveyed by the number of breaksdown in sterility is that the eggs were imperfectly sterilized.

After 35 days.

Nine spare tubes of distilled water: many eggs had hatched in all these tubes. In three of them some colourless, fuzzy looking growth has occurred, probably of fungoid origin. In six there are no signs of any growth; in a tube that was infected with *B. coli* a few larvae had reached their third instar.

Autolyzed yeast extract...	No. 1, not much further progress; some had died.
	No. 2, 2 or 3 had reached the second instar, the rest were dead.
B. coli	No. 3, larvae had reached the second or third instar; many were dead and moulds were growing, in many cases from the mouths of the dead larvae.
	No. 4, larvae in second or third instars seemingly quite healthy; this tube is now infected.
S. aureus	No. 5, only 2 living larvae, 1 in first and 1 in fourth instar; the latter was full grown; a surface mould was growing—probable bacterial infection also.
	No. 6, only 1 third instar larva was living; a mould had almost covered the surface of the tube.

	No. of Tube
B. coli	... No. 7, 4 larvae were living—all in third instar (probably infected).
	No. 8, 3 larvae were living—all in third instar (certainly infected).
S. aureus	... No. 9, 2 living larvae in third instar (certainly infected).
	No. 10, all dead in second instar (apparently still sterile).
B. coli	... No. 11, all dead; at least 2 larvae gained their fourth instar.
	No. 12, all dead in second instar (apparently still sterile).
B. coli	... No. 13, 4 larvae living in third instar; all the others died in this stage (probably infected).
Filtrate	... No. 14, a number of living larvae all still in first instar (apparently still sterile).

The history of the larvae was not continued beyond this point.

As the effect of the addition of dead cultures of bacteria to the tubes in this experiment was so much at variance with previous trials and it seemed probable that the difference was due to some variation in the eggs, an endeavour was made to check these results in the following way:

A small quantity of autolyzed yeast and dead *B. coli* culture used in this experiment were therefore added to some tubes of distilled water taken from Experiment XXIII in which eggs had been lying dormant for 100 days. These tubes were numbered 4, 5 and 6. The fluids added were autolyzed yeast and *B. coli* which had been autoclaved, and a living culture of *B. coli* as control.

To tube No. 4, about 3 % of living *B. coli* culture.
„ No. 5 „ 3 % of sterile autolyzed yeast (autoclaved).
„ No. 6 „ 3 % of sterile *B. coli* culture (autoclaved).

Result:

No. 4, 1 egg hatched within 2 minutes; 2 more within 5 minutes; 2 more within 10 minutes.
No. 5, 1 egg hatched within 15—20 minutes.
No. 6, 1 egg hatched within 15—20 minutes.

After an hour and a half.

No. 4, 8 out of 10 eggs had hatched.
No. 5, 1 „ 9 „ „
No. 6, 1 „ 11 „ „

These figures include the eggs hatched within the first 20 minutes.

A small quantity of living yeast cells from a wort agar culture was then transferred by platinum loop to tube No. 5, the remaining eggs hatched within 15 minutes; all of 8.

About 3 % of a living culture of *B. coli*, in broth, was added to tube No. 6; 1 egg hatched within 3 minutes; another followed within 5 minutes and 4 more within 15 minutes = 6 out of 10.

The failure of 4 eggs to hatch was probably due to their being dead.

EXPERIMENT XXV.

Yeast and yeast extracts.

Eggs sterilized by a slight variant of Method *c* were transferred by pipette into tubes containing 6 c.c. sterile distilled water.

The number of eggs in each tube varied between about 25—50.

On the 16th day an examination of tubes showed:

No. 1,	3 eggs hatched.	Larvae dead.
No. 2,	3 „	„
No. 3,	5 „	„
*No. 4,	none hatched.	
No. 5,	6 eggs hatched.	Larvae dead.
No. 6,	2 „	1 larva dead and 1 living.
No. 7,	3 „	1 „ „ 2 „
*No. 8,	none hatched.	
No. 9,	1 egg hatched.	Larva dead.
No. 10,	6 eggs hatched.	5 larvae dead and 1 living.
No. 11,	10 „	9 „ „ 1 „
No. 12,	10 „	Larvae dead.

* The number of eggs in these tubes was smaller than in the others, but more than 10.

To tubes Nos. 1, 8, 3 and 7 ·5 c.c. (= 1 in 12) of a sterile watery extract of yeast was added.

To tubes Nos. 2, 9, 5 and 10 ·5 c.c. (= 1 in 12) of sterile autolyzed yeast extract was added.

To tubes Nos. 4, 6, 11, 12 a small mass of living yeast cells from a wort agar culture was added.

After 10 *minutes.*

Living yeasts (*S. cerevisiae*):

Numbers of eggs had hatched in tubes 6—12 and 11, but only one in tube No. 4, but others followed almost immediately.

Watery extract of brewers' yeast:

2 hatched in No. 3, a number in No. 1, none in No. 8, and only 1 in No. 7.

Autolyzed extract of brewers' yeast:

Numbers hatched in tubes Nos. 2, 9, 5, and 10.

After 45 *minutes.*

Living *S. cerevisiae*:

Numbers, probably most, of the eggs had hatched in Nos. 6, 12, and 11; a few in No. 4. The numbers were too large for accurate counting, but one got the impression that a smaller proportion of eggs have hatched in No. 4 than in the other tubes.

Autolyzed extract of brewers' yeast:

Hatching was general in tubes Nos. 2, 9, 10 and 5.

Watery extract of brewers' yeast:

None had hatched in No. 8, only 2 in No. 3; a good proportion, probably one-third, had hatched in No. 1; only 3 in No. 7 out of a large number (probably quite 50 eggs).

Tube No. 10 was infected from No. 4 to test the effect on larval growth.

After 18 *hours.*

The hatching is now general in all the tubes to which living yeasts or autolyzed yeast extract was added.

Living yeasts (*S. cerevisiae*):

The larvae in tubes Nos. 4, 6, 11 and 12 were distinctly in advance of those in tubes Nos. 2, 5, 9 and 10, to which autolyzed yeast extract was added.

Tube No. 10, inoculated from No. 4, showed no advantage to larval growth as yet.

Watery extract of brewers' yeast:

Tubes Nos. 1, 3, 7 and 8 do not show consistent results. In No. 1, 8 eggs had hatched—a fair proportion of the eggs present (about 22 to 25). In No. 3, only about 3 eggs have hatched out of some 20—25. In No. 7, 10 have hatched (about half the eggs). In No. 8, only 3 eggs have hatched out of 15—20.

After 36 *hours.*

Watery extract of brewers' yeast:

Sterility had broken down in No. 7 and hatching was general. In No. 1 no more eggs had hatched. In No. 3 hatching was general, yet there is no evidence of infection. In No. 8 only 3 eggs had hatched.

Living yeasts (*S. cerevisiae*):

In these tubes the growth of the larvae is greatest in those which contain the fewest larvae.

Autolyzed extract of brewers' yeast:

The larvae in tube No. 10 (infected) show no advantage as yet over those in Nos. 2, 5 and 9.

After 4 *days.*

Watery extract of brewers' yeast:

Tube No. 1, tests show that this tube is still sterile; many eggs remain dormant.

,, No. 7 (infected), larval progress has been rapid.

,, No. 8, sterility broke down shortly after the previous note; all the eggs have now hatched.

,, No. 3, hatching had been very general in this tube, but it still remains sterile.

Autolyzed extract of brewers' yeast:

Tube No. 9, a bacterial growth was in progress and larval development was rapid.

,, No. 2, had been infected; the larvae were progressing.

,, No. 5, remained sterile; larvae showed scarcely any progress.

,, No. 10 (infected), larvae showed varied progress; a few were well grown in third instar, others were still small in first or second instar.

After 18 *days.*

Watery extract of brewers' yeast:

Tube No. 1 (sterile), none of the larvae had passed their first moult.

,, No. 3 (sterile), none of the larvae had passed their first moult; all dead but three.

,, No. 8 (infected), varied growth in second, third and fourth instar.

Tube No. 7 (infected), larval growth had slowed down; none had yet reached their fourth instar.

Autolyzed extract of brewers' yeast:

Tube No. 5 (sterile), larvae were all still in first instar.

„ No. 2 (infected), larvae in various stages of growth in second and third instar.

„ No. 9, all killed by vigour of bacterial growth.

„ No. 10 (infected), in third and fourth instar.

Living yeasts (*S. cerevisiae*):

The larvae in these tubes had been given more yeasts. In the tubes where they were most numerous they were in third and fourth instar. In tubes Nos. 11 and 12 where fewer larvae were present a few adults had already been bred and the remainder were either full grown larvae or pupae.

After 42 days.

Watery extract of brewers' yeast:

Tube No. 1 (sterile), all were dead; none past second instar.

„ No. 3 (sterile), 3 or 4 living in second instar, the rest dead in first instar.

„ No. 8 (infected), nearly all dead in various stages.

„ No. 7 (infected), all dead but one in its third instar.

Autolyzed extract of brewers' yeast:

Tube No. 5 (sterile), a few had reached their second instar.

„ No. 2 (infected), but little, if any progress since 18th day.

„ No. 10 (infected), 1 adult reared, 1 pupa, many full-fed larvae.

Living yeasts (*S. cerevisiae*). No food since 18th day:

Tube No. 4, all dead, but 1 in third instar.

„ No. 6, all dead, but 1 in third instar.

„ No. 11, 2 adults reared; remainder dead in various stages.

„ No. 12, all dead, but 1 in fourth instar.

The history of the larvae was not continued beyond this point.

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Mitchell (1907). *Mosquito Life,* p. 145. G. P. Putnam and Sons, New York and London, 1907.

[1] Both quotation and record are taken from Howard, Dyar and Knab (1912), Vol. I. p. 281. We have been unable to refer to this book in London. There is no reference to it in the *Zoological Record.*

OBSERVATIONS ON *ENTAMOEBA GINGIVALIS* FROM THE HUMAN MOUTH, WITH A NOTE ON THE TRICHOMONAD FLAGELLATE *TETRATRICHOMONAS BUCCALIS* N. SP.

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(With Plates XX—XXII.)

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Introduction.

The claim made for the first time by Barrett and Smith (1914*a*) some two years and a half ago, that the causative agent of Pyorrhoea Alveolaris is the *Entamoeba gingivalis* led the writers to enquire into the question; the results of which enquiry are set forth in the following paper.

From the first we were not convinced that the mere association of an organism with a particular set of circumstances in a certain percentage of cases is proof that we are dealing with cause and effect. Nor, when yielding to the inhibitory effects of some specific reagent the organism disappears, and *pari passu*, the abnormal circumstances also show a tendency to disappear, can we yet agree that the association of one with another as cause and effect has been definitely established.

So far, all attempts to reproduce the disease by inoculation of healthy tissues with discharge containing amoebae have failed. In regard to treatment, if we grant that certain cases do well with emetine after having proved refractory to all other methods, it remains still to be proved that the improvement is due to the amoebicidal action of the drug and not to some other possible effect, such (as has been suggested) as its bactericidal action or its possible beneficial effect upon the phagocytic function of the leucocytes.

It is unnecessary for us in this paper to discuss the nomenclature of *Entamoeba gingivalis* nor need we discuss the historical aspect of the question; all this has been done quite recently and very thoroughly in one or two papers dealing with this problem, *e.g.* Smith and Barrett (1915 *b*) and Craig (1916).

We have contented ourselves with two principal lines of attack on the problem. First, a careful investigation into the structure of the amoeba by exact cytological methods, particular attention being directed to the nature of the inclusions, and, second, an examination of a number of mouths, some healthy, others showing a variety of pathological conditions.

We have not attempted to investigate the conditions influencing the trophic existence of the amoeba, as for example, the effect of the saliva as regards the amount secreted and its chemical composition, or the influence of the fermentative changes proceeding in the buccal cavity.

Methods.

(*a*) *Observation on the living organism.*

For making observations on the living amoebae we have employed the warm stage with success.

We have found it convenient to make our preparations for examination under these conditions as hanging-drops on cover-slips placed over cavity slides and sealed with wax. By employing this method it is possible to lift the cover-slip after making observations and fix off the preparation at any desired moment.

In making cover-slip preparations we have taken a small quantity of material from the mouth, whether pyorrhoea pus or food or other *débris* by means of a sterile needle or capillary pipette and have teased it up with a platinum loopful of a diluting solution.

The solutions used for this purpose have been normal salt solution, saliva, salt-citrate solution of the strength recommended by Minchin[1] for the examination of trypanosomes, and salt-citrate solution to which 15 % white of egg has been added. By the use of the last-mentioned solution it is possible to get very good film preparations on fixation, for the egg-albumen in coagulating adheres to the cover-slip and holds most of the bodies present in the film.

It is important in making hanging-drop preparations to use only the minimal quantity of diluting solution, so as to ensure a thin layer of liquid when the majority of the leucocytes and organisms will be found on the glass surface, and not on the lower surface of the drop. If too much solution is taken, not only is it very often impossible to use high powers in the examination of the organisms, for most of the latter gravitate to the lower surface of the liquid, but when the cover-slip is lifted, and dropped on to the fixative, all those bodies on the lower surface of the drop are washed off and are lost. We have found normal salt solution and salt-citrate solution with or without egg-albumen equally good as the diluting solution.

(*b*) *Fixed and stained material.*

As fixatives we have used Maier's solution which is very similar to Schaudinn's sublimate alcohol, Bouin's fluid, and absolute alcohol.

The last is especially useful for rapid work when methyl-green is used as the subsequent stain. Maier's and Bouin's solutions appear to give equally good results but we have used Maier's solution most frequently.

After fixing we have followed the usual procedure for the manipulation of wet films, washing out the fixative and finally bringing the preparation into the desired staining solution. It may not be out of place at this point to call attention to the utter uselessness of dry-film preparations for the exact cytological study of mouth amoebae or of any other protozoa. We have seen particulars given for the making of such dry-films by the usual rapid bacteriological methods and recommending the use of certain rapid stains such as thionin-blue.

[1] 1 grm. sodium citrate and 1 grm. sodium chloride dissolved in 200 c.c. distilled water.

Such methods may be of service in diagnosing the presence or absence of the amoebae in a given preparation but even then their use and value is questionable especially if it should happen that the material contains only small examples of *E. gingivalis*. It is absolutely essential, as Craig points out, to keep the film moist during the whole of the fixing and staining processes if any adequate idea of the structure of the organisms is to be obtained.

We have found iron-haematoxylin to be the most useful stain for revealing the detailed structure of the organisms and most of our films are stained with this.

Dobell's iron-haematein[1] is a very good stain which is especially useful on account of the rapidity with which it can be used. After washing out the fixative the preparation is treated in the mordant, 1 % ferric ammonium alum in 70 % alcohol for ten minutes. It is then rinsed in 70 % alcohol and stained for ten minutes in 1 % haematein in 70 % alcohol, following which, it is differentiated in the mordant or in acid 70 % alcohol, and finally washed in several changes of ordinary 70 % alcohol. By the use of this stain one can quickly obtain well-stained preparations presenting clear pictures of the detailed structure of the organisms.

Other stains which we have employed are Jenner's blood stain, Giemsa's stain, Unna's polychrome methylene-blue, safranin and lichtgrün, Mann's methyl-blue eosin, and methyl-green. In using the last-mentioned stain we have found it best to fix the preparation in absolute alcohol for about ten minutes and then take down through graded alcohols to water, after which it is floated on a solution of methyl-green in 1 % acetic acid for a minute or so and then mounted on a slide in a small quantity of Ripart and Petit's medium and sealed round with marine glue. Since this stain has only been used to investigate the nature of the round inclusions within the amoeba and also the character of the amoeba nucleus its impermanency is a matter of no consequence.

Morphology.

(*a*) *The living amoeba.*

We are not in a position to add anything new to the published accounts of the structure, appearance, and movements of the living amoeba in its ordinary vegetative stage of existence. The recent paper by Craig (1916) deals fully and adequately with these matters.

[1] Dobell, C. C. (1914). Cytological studies on three new species of Amoeba. *Arch. f. Protist.* xxxiii. 143.

When a preparation of material from the mouth is first examined it is not at all easy to pick out the amoebae especially if there are large numbers of polymorphonuclear leucocytes present. After a short time however one's eye becomes accustomed to the field and one can then pick out from the vast numbers of round bodies certain ones which have a denser appearance and a brighter green shimmer than the rest. These are the amoebae and if they are examined under the high power it will be found that they appear quite different from the polymorphonuclear leucocytes, in which it is often possible to distinguish the lobed nucleus surrounded by finely granular protoplasm.

If examined on the warm stage an amoeba will soon be seen to send out small lobose pseudopodia of clear ectoplasm in various directions in which case it will not progress much in any given direction. One large pseudopodium may be extruded and the amoeba will then take on a "limax" like appearance (Pl. XX, fig. 1) and the movement will be chiefly one of translocation.

As already mentioned the ectoplasm is hyaline in appearance in the pseudopodia, whilst the endoplasm is coarsely granular and is made up very frequently of a large number of vacuoles, some of which appear to be empty whilst others contain inclusions of various sizes and shapes.

If a preparation showing actively motile amoebae is examined for any length of time, half to three-quarter's of an hour, on the warm stage it will be observed that as an amoeba moves about, its posterior end frequently becomes entangled in clumps of bacteria and polymorphonuclear leucocytes. The majority of these adherent bodies appear to be a hindrance to its continued progress, and the organism seems to be trying to get rid of them; the polymorphonuclear leucocytes especially seem very sticky.

On two occasions we have observed a small round homogeneous body, which we have taken to be of the same nature as one of the larger food bodies, become attached to the posterior end of an amoeba and instead of being cast off after a time, the amoeba has gradually taken the body into its endoplasm. We have never observed the intake of a polymorphonuclear leucocyte in this manner nor have we ever seen anything in our stained preparations suggestive of a whole ingested polymorphonuclear leucocyte within an amoeba.

There is no contractile vacuole.

We agree entirely with Craig when he says that in the vast majority of cases the nucleus is invisible in the living organism. On only two or three occasions have we satisfied ourselves that the round body

within the endoplasm which we have taken for the nucleus has been that structure. When an amoeba shows numerous round inclusions, as is often the case, it is impossible to say that any particular round body in the endoplasm is the nucleus.

(*b*) *The stained organism.*

When stained with iron-haematoxylin the ectoplasm of the amoeba stains grey and appears finely granular in structure in protruding pseudopodia. The endoplasm is often sharply marked off from the ectoplasm and appears to be somewhat coarsely granular in structure and frequently shows an alveolar appearance. Usually one finds inclusions of various shapes and sizes within the endoplasm and these are often present in sufficient numbers to obscure the structure of the endoplasm.

The nucleus has been fully and accurately described by Craig who deals also with the differences and similarities between it and the nuclei of *Entamoeba coli* and *Entamoeba histolytica*. It consists essentially of a circular or oval nuclear membrane separated by a clear zone from a small centrally situated karyosome. The membrane seems to be impregnated with a certain amount of chromatin for it stains deeply with iron-haematoxylin and also frequently bears granules or small thickenings of chromatin on its inner side. The karyosome may appear to consist of a single body homogeneous in structure and this is most commonly found, or it may very frequently appear to be made up of three or four distinct granules. In several of our preparations a karyosome appears to be absent and the space within the nuclear membrane is taken up with a net-work of linin fibres on which small granules of chromatin are irregularly disposed. It is possible that such appearances may represent stages in the break up of the chromatin prior to nuclear division, though none of the amoebae show anything which can be interpreted as mitotic division figures.

The nucleus is extremely poor in chromatin, and only stains feebly with chromatin stains such as safranin, methyl-green, polychrome methylene-blue. There is thus a very marked contrast in appearance between it and the round or oval inclusions, many of which are coloured intensely with the stains just mentioned. Pl. XX, fig. 2 is a photomicrograph of an organism stained with safranin and shows how feeble is the contrast between the cytoplasm and the nucleus.

Wenyon (1907) speaks of the paucity of chromatin in the nucleus of *Entamoeba muris*, and in this connection also we may note that a

section of an intestinal ulcer showing *Entamoeba histolytica* which was kindly lent to us by Dr J. H. Ashworth, showed the cytoplasm of the amoeba stained blue with methylene-blue whilst the nucleus was stained pink with eosin. One generally thinks of nuclei as basophilous and not eosinophilous.

Nuclear division must take place but so far we have not found any organisms which show any stage of the process.

We have found one bi-nucleate amoeba which is shown in Pl. XX, fig. 6, and on one film we were fortunate in obtaining a late stage in the fission of an amoeba, shown as a photomicrograph in Pl. XX, fig. 7.

So far, we have not been successful in finding either the cystic or the pre-cystic stage described and figured by Craig.

Our measurements of the amoeba and the nucleus agree on the whole with those given by previous workers. One of the smallest forms we have found is that shown in Fig. 5 and measures $7{\cdot}5\mu$ in its longer axis. The organisms vary from this up to about 25–27μ when in an extended condition. We have not found any of the large forms mentioned by Smith and Barrett reaching 60μ in length. The nucleus measures from about $2{\cdot}5\mu$ to 4μ in diameter.

(*c*) *The Food of* Entamoeba gingivalis.

The importance of determining the nature of the food of *E. gingivalis* need not be emphasised here except to point out its relevancy to the question of the possible pathogenicity of the organism.

Reference to the literature dealing with this subject yields no definite information on the point. Doflein (1911, p. 595) says that the amoeba feeds chiefly on leucocytes and bacteria, whilst Hartmann (1913, p. 641) says that the food consists of bacteria of the mouth cavity, but chiefly of leucocytes and leucocyte remains.

These statements appear to be based largely on the remarks of von Prowazek and Leyden and Loewenthal on the subject. Prowazek (1904) says that *E. buccalis*, which is undoubtedly the same organism as *E. gingivalis*, lives among leucocyte masses, and takes in this food (presumably the leucocytes) by ectoplasmic engulfment. He goes on to describe the process of digestion, as revealed by the aid of neutral red, and the throwing out of the undigested nuclear remains.

Leyden and Loewenthal (1905, p. 9), in speaking of the amoebae found by them on the surface of a cancer inside the mouth say that the food appears to consist chiefly of leucocytes, with whose remains they are frequently completely filled, whilst bacteria are seldom found in

food vacuoles. They go on to state that the food bodies in the amoebae exhibit the staining reactions of nuclear substance with haematoxylin and iron-haematoxylin.

We may usefully point out here what has been mentioned already, that in none of our observations either on the living organisms or on stained material have we found any evidence whatever in support of Prowazek's statement that the amoeba ingests leucocytes, if by this word is meant polymorphonuclear leucocytes.

Smith and Barrett (1915 *a*, p. 166) say that in the endosarc there are many digestion vacuoles in which globular detritus of leucocytic nuclei and red blood cells are commonly found along with bacteria.

Craig (1916, p. 230) says that not all the ingesta are of leucocytic origin, and puts forward the suggestion that those round bodies which stain deeply with iron-haematoxylin and are surrounded by a clear zone, represent some form of yeast or protozoan organism, and are not of leucocytic origin. He also mentions that it has not been his experience to observe ingested red corpuscles in the endoplasm, even where the surrounding medium contains red blood cells.

Colyer (1916, p. 52) gives points for and against the amoeba being the cause of the disease. Among the points in its favour he speaks of "its power to phagocyte red cells, a power not possessed to any degree by free-living amoebae."

From this short account of previous work on the subject it will be seen that there is no agreement as to the real nature of the ingesta, except that most of the authors mention leucocytes or leucocytic remains, whilst two consider that red cells may be among the ingesta. The word leucocyte has a wide connotation, and it was therefore necessary for us to carry the matter still further, and obtain as far as possible accurate information on this point. We have made a careful attempt to determine the real nature of these bodies, because we realised that upon the elucidation of this point depended, to a large extent, the answer to the question whether or no *E. gingivalis* is a true parasite of pathogenic importance.

The ingesta within the amoeba are seen to consist principally of two constituents, bacteria and larger bodies of various shapes and sizes, but most frequently round or oval in outline. These bodies are by far the commonest constituent of the ingesta, though one may occasionally encounter amoebae without any food bodies at all. Again, other amoebae are found showing only bacteria within them (Pl. XX, fig. 3), either lying singly or in groups within vacuoles, and one not uncommonly

finds organisms in which both bacteria and the larger bodies occur together, as shown in Pl. XX, fig. 4. The round bodies present a greenish appearance in the live amoebae, and when stained appear to be almost homogeneous in structure. They frequently show a narrow clear zone around them, separating them from the inner limit of the vacuole, but as often seem to occupy the whole area of the vacuole.

With iron-haematoxylin, the majority of them stain an intense black whilst others exhibit various degrees of blackness, and some are only faintly stained.

The black coloration with iron-haematoxylin suggests that they are composed of a chromatinic substance, a fact which is confirmed by the use of other stains. Safranin stains the bodies bright red, Unna's polychrome methylene-blue stains them deep blue, methyl-green colours them green, whilst with Giemsa's stain they are tinted a bluish purple or purple, and Jenner's methylene-blue eosin stains them blue or greenish blue.

All these stains, therefore, show that the bodies are rich in chromatin, and that they are probably derived from nuclear material. They also definitely rule red blood cells out of the question.

This is important, for it shows that the bodies are not of the same nature as the ingesta of *E. tetragena*, in which red blood corpuscles are frequently found.

Nor are whole polymorphonuclear leucocytes ever to be found within the amoebae, although endothelial cells showing ingested polymorphonuclear leucocytes are frequently observed in material from pyorrhoea-pockets or from inflamed areas of the gums (Pl. XX, fig. 8).

In size the bodies vary from about 1 μ to 4 or 5 μ in diameter, our measurements agreeing with those given by Craig.

Having satisfied ourselves of the chromatinic nature of the deeply staining bodies we set out to discover their origin. It was evident that they were of common occurrence in the mouth, and were not confined to pyorrhoea pockets although they were to be found in amoebae from these situations as well as from any other part of the gums or necks of teeth. We were not satisfied that they were derived from polymorphonuclear leucocytes, for none of these ever showed nuclei of the homogeneous type and having the same staining reactions as the round bodies. We were, therefore, driven to make as thorough a search as possible for the source of the round bodies.

Careful examination of our films carrying amoebae showed that, scattered about amongst the innumerable bacteria and leucocytes,

there were here and there small bodies having exactly the same shape, size, and general appearance as the ingesta of the amoebae. It was clear that if we could satisfactorily explain the nature and origin of these bodies we should be in a position to clear up the question of the ingesta of the amoeba.

The clue to the solution of this problem was obtained when it was observed that on one of our films certain of the bodies within the amoebae, and scattered about in the film, possessed small fragments of cytoplasm attached to the deeply staining nuclear substance of which they were composed (Pl. XXI, fig. 13). It was further noticed that occasionally the bodies occurred in groups of two or three (figs. 13 *a*, *b* and *c* and 14 *a–m*), and this, together with the occurrence of the cytoplasmic remains attached to the bodies suggested that they were produced by the disintegration of some form of polymorphonuclear leucocyte.

It was known that in the saliva there occurred the salivary corpuscles, which could be observed undergoing disintegration and dissolution, when saliva was examined on the warm stage.

Film preparations were therefore made from saliva and from the tonsils, from which the salivary corpuscles are poured out, and were fixed and stained in exactly the same way as the films for amoebae.

Examination of these films, salivary and tonsillar, revealed salivary corpuscles in an almost endless variety of degeneration and disintegration, and it was found that round bodies exactly similar to the ingesta of the amoebae and to those scattered about on the amoebae films were present on these films also.

It was at once apparent that the bodies in question were produced from the nucleus or nuclei of degenerated and disrupted salivary corpuscles.

The latter were found to present a variety of nuclear appearances; some were mononuclear, others showed all kinds of transition stages between the purely mononuclear and the polymorphonuclear condition. The size also varied greatly. Those showing the polymorphonuclear condition had a very different appearance especially in the lobes of the nuclei from the true phagocytic polymorphonuclear leucocyte which is also very common in the mouth. The photomicrographs, shown in Figs. 9 and 9 *a*, illustrate the differences very well[1].

[1] *N.B.* It is not within the province of this paper to go into the question of the evolution of salivary corpuscles within the body nor into their relation to the leucocytes of the blood. Reference should be made to the writings of Weidenreich (1908 and 1911) for discussion of these subjects.

Our films show that when the salivary corpuscles undergo dissolution in the saliva and in any situation in the mouth, their cytoplasm becomes coarsely granular in appearance, and is finally ruptured and thrown off from the nuclei which may remain with fragments of cytoplasm attached to them, or may be left completely naked. The nuclei also stain in a great variety of ways. Some of them appear to swell up and only stain faintly, others seem to remain normal in size, and some apparently shrink a little and are stained an intense black with iron-haematoxylin. The majority of them appear to become practically homogeneous in structure.

Not all the nuclear lobes of a given corpuscle behave in the same way, one portion may be pale and the other or others quite black. The series of drawings shown in Pl. XXI, fig. 14 *a–m* gives a small selection of disintegrating salivary corpuscles and their products taken from films of saliva and tonsillar smears.

A comparison of these with the accompanying drawings of amoebae with their ingesta will at once show the identity of the bodies in question. It would be easy to multiply the drawings indefinitely and so accumulate still more evidence in support of our suggestion, but we think that the figures given are sufficient to illustrate our point.

Salivary and tonsillar smears stained by other methods than iron-haematoxylin, as for example with Giemsa's or Jenner's stains, also point conclusively in favour of the explanation we have advanced.

It is easy to see how, with the extrusion of salivary corpuscles and their subsequent disintegration always going on, a supply of their nuclear remains can accumulate in the mouth, on the gums or at the bases of the teeth, in fact at any point where a suitable lodgment can be found, and how the amoebae are continually furnished with fresh supplies of food by this means.

Our explanation of the nature of these bodies is at once simple and feasible. It is, further, easy to understand the extreme prevalence of the bodies among the ingesta of the amoebae, and at the same time shows that the organism is of no pathogenic significance, but is on the other hand a scavenger of naturally occurring waste nuclear material together with bacteria occurring in the mouth. It may therefore be considered as a useful rather than a harmful organism.

As has been mentioned earlier, previous investigators have suggested the leucocytic nature and origin of the ingesta under discussion but the point which we make and one that has not been suggested before is

that the bodies are the product of certain definite leucocytes, namely the salivary corpuscles.

Craig's suggestion mentioned above, that the black bodies surrounded by a clear zone are some protozoan or yeast-like organism, receives no support from our observations, and seems to us unnecessary. We do not wish to assert that yeasts may never be ingested by the amoebae, for we have found a few small yeast-cells on smears made from the healthy gums of the child of one of us (T. G.). A few amoebae are present on these films, and show the usual round deeply staining bodies amongst the ingesta but not yeasts. The bodies under discussion are also quite distinct in appearance from the yeast-cells which may frequently be found ingested by *Entamoeba coli*, in smears made from faeces. A drawing of one of these is shown in Pl. XXI, fig. 15.

Description of Cases.

1. Child, male, aged 2. Healthy mouth and gums. Smears taken from gum margin. Amoebae present.

2. Girl, aged 5. Sent by Dr Waller from the Birmingham General Hospital to the Dental Hospital, with the extremely rare condition in the deciduous dentition of the progressive loosening and loss of temporary teeth, one after another in both jaws, the teeth being shed with no absorption of the roots whatsoever. The disease appeared to commence in the gum, extending to the bone of the socket and the periodontal membrane, being finally cast off with the total destruction of the latter structure. The condition is analogous to pyorrhoea alveolaris in the permanent dentition.

The amoeba was found in the discharge squeezed from between the gum and an affected tooth; and amongst the *débris* collected from the end of the root of the lower right canine which was loose enough to be picked from its socket with the fingers.

3. Boy, aged 13. Teeth in a shocking state of irregularity, and very carious. He had been wearing appliances for the regulation of teeth for some time. The mouth was very badly kept, *materia alba* accumulating over the buccal and labial surfaces of the teeth producing very rapid and extensive caries. The gums were inflamed on the buccal and labial surfaces of both jaws but no pockets existed. Amoebae not present.

4. Girl, aged 10. Mouth well-kept, gums healthy. An orange-stained deposit on the labial surface of the erupting lower right canine was examined, but no amoebae were found.

5. Boy, aged 13, with inflammatory hypertrophy of the gum on the labial aspect of the maxillary incisors. Material was collected from beneath the enlarged gum festoons and was found to be very rich in amoebae. The hypertrophied tissue was removed, fixed in Bouin's solution and embedded in paraffin. Serial sections were cut, stained by a variety of appropriate methods, and carefully examined for the presence of amoebae with a negative result.

6. Girl, aged 16. Teeth in fairly good condition but the interdental gum papillae were somewhat enlarged and inflamed, due to the retention of food *débris*. Films made from the *débris* in the "trough" between the papilla and the tooth showed the presence of amoebae.

7. Female, aged 21. Teeth well-kept and mucous membrane of the mouth generally healthy, but the margin of the gum around the upper right maxillary first molar was irritated by a small collection of salivary calculus, and had reached a stage of chronic inflammation. Films made from the material beneath the inflamed gum, and others made from healthy gum showed no amoebae.

8. Male, aged 31. Mouth very well kept. Singularly free from dental caries. Case of true pyorrhoea alveolaris affecting chiefly the upper and lower incisor regions, the left lower molars, and to a slight extent the left maxillary canine (lingual aspect only) and premolars. In the infected areas the gum had a tense, glistening appearance and was of a deep bluish-red colour, deepened over the region of the pockets, so that one could distinguish by the colour of the gum the positions where the greatest amount of bone destruction had occurred. The interdental papillae were retracted; not removed by ulceration as one sees in some conditions, but drawn down tightly over the interdental bony septum, becoming more and more retracted, as more and more bone was destroyed. From the pockets, a fairly copious amount of pus could be expressed at almost any time; and on the roots of the teeth corresponding to the position of the retracted gum a deposit of hard black calculus was found, which in some places extended along the root for a short distance into the pocket. Films were made from the pus from around the upper incisors, but we did not detect the presence of the amoeba until we had examined upon the warm stage some of the material from the mandibular incisors. At no time were the amoebae very numerous, but the most favourable moment for them was in the first expressed bead of pus which would be contaminated with a certain amount of *débris*.

We cannot agree with the statement that the amoebae are most numerous at the bottom of a pyorrhoea pocket. Our experience proves

the contrary, namely, that if precautions are taken to avoid contamination with the superficial *débris*, material collected from deep pockets is less rich in amoebae than that collected from a more superficial area.

9. Male, aged 34, with mouth in a very unhygienic condition. The buccal and labial surfaces of the teeth were badly discoloured, some of the posterior teeth being coated with salivary tartar, whilst all the remaining molar and premolar teeth had a rim of hard calculus at the cervical margin on both the buccal and lingual surfaces. There was a marginal gingivitis in both jaws, but no suppuration could be detected except around the left mandibular second molar, where it was very slight. The interdental papillae were reduced in size and inflamed. There were no distinct pockets, but in places the alveolar border and gum had shrunk, leaving the neck or the root of the tooth exposed.

The amoeba was found on both the salivary calculus and the rim of hard dark calculus at the gum margins; amongst the white deposit on the buccal surfaces of the molars, and in the *débris* beneath the inflamed gum margins.

10. Male, aged 37, emaciated, thin and anaemic, suffering from chronic rheumatism. He was sent from a General Hospital for dental treatment. His mouth was in a very neglected state, with several badly broken-down septic roots, the buccal surfaces of the remaining posterior teeth covered with salivary calculus, and the usual rim of hard dark tartar associated with marginal gingivitis at the necks of the anterior teeth, and on the lingual aspect of the necks of the posterior teeth. The marginal gingivitis was very pronounced. Hanging-drop preparations and stained films from both kinds of calculus, and from the matter expressed from the gum margins showed abundant active amoebae.

11. Female, aged 40. Recession of gum around buccal roots of left maxillary first molar. No pus present, and no pockets. Films made from *débris* on the exposed roots showed amoebae.

12. Female, aged 38. Recession of gum from buccal and distal surfaces of left upper maxillary second molar due to presence of calculus, but there was no visible suppuration. Stained films showed the presence of amoebae.

13. Male, aged 43. Pyorrhoea affecting all remaining maxillary teeth including the incisors, canines and second molars. There was recession of the gums all round the teeth and the formation of pockets on the lingual aspects of the incisors and canines and the mesial surfaces of the molars. The whole of the palatal surface of the gums, periosteum, and that between the canines and second molars was in a

"*boggy*" condition to the touch. Suppuration was not constant and had been kept fairly well in check by periodical local treatment. Occasionally a pocket would get infected, giving rise to acute inflammation and the formation of a local abscess. Amoebae present.

14. Male, 49. Similar to "13." Films also showed presence of the organism.

15. Male, aged 43. Films made from the material collected in the "trough" existing between the normal healthy interdental gum papilla and the tooth showed the presence of amoebae, flagellates and numerous active phagocytic leucocytes.

16. Male, 36. Recession of the gum on labial surface of root of the first left lower incisor exposing more than one-third of the root. Material collected from the exposed root contained amoebae, as did also material from a rather deep "trough" existing between the distal surface of the mandibular second left molar and the gum.

17. Female, aged 30. Inflammation of gums chiefly affecting the labial and buccal aspects and the interdental papillae of both jaws. There was some recession of the gums about buccal surface of right premolars and first molar, but no deep pockets were to be found and there was a tendency to hypertrophy of the interdental papillae rather than resorption. The whole of the affected gum surface was of a deep red colour, bleeding freely to the slightest touch and very painful. Material collected from between the inflamed gum and the tooth surface contained numerous amoebae.

18. Male, 52. Leucoplakia of the cheeks, affecting both sides. On right side it seemed to extend from inflammatory condition of gum around second mandibular molar. Films made from about the molar, and the surface of the cheek did not show any amoebae.

19. Male, 55. Chronic condition of the tongue in which the sides of the anterior third and the tip were liable to recurrent attacks producing painful, raw patches. No amoebae.

20. Male, 50. All teeth were in a perfect condition except right maxillary first molar which was missing. The mouth was exceedingly well kept and the gums generally speaking were in a good condition, with the exception of slight recession of the buccal margins about the premolar and molar teeth in both jaws and some evidences of an inflammation of the interdental papillae here and there.

Films made from the "troughs" existing between the interdental papillae and the teeth showed the presence of amoebae in the case of both healthy and unhealthy papillae.

21. Male, 38. So-called dental ulcer on the lingual aspect of the left mandibular gum, caused by the chafing of a denture. Film made from its surface gave negative results so far as amoebae are concerned.

22. Male, 30. A case of marginal gingivitis commencing in the interdental papillae, producing rapid sloughing of the latter and extending along the gum margins. Amoebae very numerous.

23. Male, aged 28. Right mandibular second molar which had been capped with gold became very painful and loose, with a good deal of inflammation in the gum around it. After extraction the *débris* collected on the surface of the gold and on the roots was examined but no amoebae were found.

24. Male, aged 44. A "bridge" attached by gold caps to the left maxillary first premolar and the third molar on the same side, was extracted together with the abutment teeth, on account of the loosening of the latter by the gradual destruction of the bone and resorption of gum. No discharge was present. *Débris* from the roots, from the surface of the gold caps and from under the surface of the gold "bridge" was examined and in no case were amoebae found.

Summarising the results, we have found *Entamoeba gingivalis* present in the majority of cases of pyorrhoea, but not in all. It is also present in most cases of gingivitis not associated with alveolar absorption, although here also in some of our cases the organism is absent.

In some mouths with local evidences of recession of the gum associated with alveolar absorption, the amoeba was found in the diseased positions, and also in the healthy positions in the same mouths. We have found it in a case of marked hypertrophy of the gum as well as in cases of rapid sloughing of the margins of the gums and the interdental papillae.

The organism was present in the healthy mouth of a child of two years old, and also in that of a man of 43. It was not present in an unhealthy mouth of a boy, aged 13, with very irregular teeth, most of which were rapidly succumbing to dental caries; and whose gums were also inflamed and bled easily (No. 3). Neither was it present in an orange-stained deposit on the labial surface of a lower canine in a child of 10 (No. 4). In three other pathological conditions in the mouth (Nos. 18, 19, 21) we have not found the organism, although cases of aphthous and ulcerative stomatitis and glossitis examined by Lynch (1915) gave positive results.

We conclude from our examinations of the above cases that *Entamoeba gingivalis* is not the cause of pyorrhoea alveolaris, since,

although common in the deposits around the teeth in this condition, it is by no means always present. Further, its presence in other pathological conditions of the oral muco-periosteum, and also under certain circumstances in healthy mouths does not support the claims of Smith and Barrett and others; but on the contrary seems to suggest that it is dependent upon a factor common to all these cases. That factor is, in our opinion, the existence of a "trough," or an interdental space, or a pocket, or a rough surface such as that afforded by an accumulation of tartar, or other place inaccessible to the natural cleansing agencies of the mouth, where food particles and cellular *débris* and bacteria can collect, and where the amoeba exercising its (in our view) beneficent function of scavenger of dead nuclear matter and bacteria, would find plenty of sustenance.

The fact that it is not found in all such cases (see Nos. 3, 4, 7, 23 and 24) we are not at present able to explain; possible explanations are: (1) The influence exerted upon the amoeba by the reaction of the saliva. (2) The kind of food accumulating, whether proteid or carbohydrate were in excess, would determine the kind of change going on in the accumulation, *i.e.* putrefactive (alkaline) or acid-production. (3) The variations in the mouth flora[1]. Cases 3 and 4 in which the *débris* collected from the tooth surfaces was strongly acid seem to suggest that the mouth amoeba has no great fondness for an acid medium.

Conclusion.

Bringing together the evidence derived from the two sources, viz. (1) the cytological investigation into the nature of the ingesta of the amoeba, and (2) the examination of a variety of oral conditions, as to the relation of *Entamoeba gingivalis* to Pyorrhoea Alveolaris, we find that the first leads us to the conclusion that the organism so far from being a destroyer of healthy tissues in the mouth is in reality a devourer of waste nuclear material derived from disintegrated salivary corpuscles together with bacteria and is therefore most probably a useful scavenger, whilst the second leads us to the conclusion that the amoeba may be found in healthy and unhealthy mouths and is especially likely to be found in situations where there is the possibility for the accumulation of food *débris*.

Our general conclusion is that there is no evidence to show that *E. gingivalis* is the cause of disease.

[1] These of course must depend largely upon the nature of the oral secretions and the character of the food.

A NOTE ON THE TRICHOMONAD FLAGELLATE *TETRATRICHOMONAS BUCCALIS* N. SP.

By T. GOODEY.

In several of the recent papers dealing with *Entamoeba gingivalis*, mention is made of the occurrence in preparations from the mouth of a trichomonad flagellate. One writer, Lynch (1915), names it *Trichomonas vaginalis* evidently identifying it with the flagellate bearing that name. Other writers adopt the course followed by the text-books of including the organism under the name *T. hominis* or *T. intestinalis* and so identifying it with the human intestinal form. The only published figures showing the appearance of the organism are those attributed to v. Prowazek which are however very unsatisfactory. In the light of these considerations the following note is put forward as an attempt to present some accurate information as to the appearance and structure of the organism.

The flagellate was found fairly plentifully in material consisting of food *débris*, etc., removed from between the lower incisors of one of the cases examined by us. Hanging-drop preparations were made and examined on the warm-stage, and though at first the flagellates did not appear to be numerous, after a short time they were found in fairly large numbers, actively moving about in the drop.

Their movements were very rapid, and it was impossible to determine anything concerning the number and movement of the flagella, neither could it be ascertained whether an undulating membrane was present.

The posterior end was frequently seen to be much drawn out into a long tapering point, and the organisms often displayed great amoeboid activity, sending out pseudopodial extensions, both anteriorly and posteriorly.

The hanging-drops were fixed and stained so that the organisms could be studied in detail.

It was found that in staining with iron-haematoxylin the flagellates differentiated very quickly, so that by the time the leucocytes and amoebae on the films were differentiated, the flagellates were practically decolorised entirely. It was therefore necessary to differentiate for a short time, and by this means, satisfactorily stained flagellates were obtained.

When properly fixed and stained it is found that the body of the flagellate is longer than it is broad, varying in length from about 7 μ to 12 μ, and that the protoplasm of which it is made up is alveolate in structure.

Small bacteria are frequently to be seen lying in the protoplasm of the body. A mouth or cytostome could not be distinguished in any of the numerous organisms examined, but one may be present nevertheless. It is highly probable that the flagellate feeds by the ingestion of bacteria by means of pseudopodial activity, when undergoing amoeboid movement. Pl. XXII, fig. 21, showing a flagellate with numerous ingested bacteria in a much extended amoeboid condition, supports this suggestion.

The nucleus, which is oval or ellipsoidal in shape, lies towards the anterior end of the body. It seems to stain rather diffusely, and shows, so far as our preparations reveal its structure, no karyosome. Occasionally, a few rather more deeply staining areas can be distinguished in it.

In front of the nucleus there is a distinct round or oval blepharoplast, which is connected with the nucleus by means of a short rhizoplast.

The flagella are four in number, not three as in *Trichomonas intestinalis*, and arise from the blepharoplast. They are rather long and delicate, all appearing to be of about the same length, and stain faintly.

There is an undulating membrane which takes origin in the blepharoplast, and extends along one side of the body, to about two-thirds or three-quarters the length of the latter. There does not appear to be a free posterior flagellum to the undulating membrane, and in this respect it differs from *Trichomonas intestinalis*, and many other species of the same genus.

A chromatinic basal rod ("parabasal body" of Kofoid) appears to be present in connection with the undulating membrane, though it varies greatly in its extent and staining reactions. In one or two cases it appeared to follow the edge of the organism, whilst in others it seemed to be curved on to the surface, as shown in Pl. XXII, figs. 17 and 18. In one case it was represented by a row of granules, whilst in several it was not possible to distinguish anything at all corresponding to it, as shown in Fig. 19.

An axostyle is present, and extends from the region of the nucleus, where it seems to be somewhat expanded, in a posterior direction and frequently terminates in a sharp spike outside the end of the body, as in Pl. XXII, figs. 17–20.

In the amoeboid form shown in Fig. 21 it is seen as a straight rod. It stains rather more deeply than the cytoplasm of the body, and does not stand out as clear, almost unstained organ, as represented in the drawings of many species of Trichomonas, but resembles more the appearance of the axostyle figured by Mackinnon (1913) in *Tetratrichomastix parisii*.

The question of the naming of this organism presents certain difficulties.

It is, without doubt, the same trichomonad as that figured by Doflein (1911, p. 493) and by Jollos (1913, p. 693), the figures being taken from Prowazek. The finely tapering tail portion and the appearance of the undulating membrane prove this. Doflein calls it *Trichomonas hominis*, and Jollos *T. intestinalis*; each identifying it with the trichomonad from the intestines, and explaining its different appearance from that form as being due to the influence of different environmental conditions. Whether this is a sufficient explanation of the morphological differences presented by the mouth and intestinal forms is, in the writer's opinion, rather doubtful. The great variety of shape exhibited by the mouth form, as compared with the fairly constant oval or pyriform shape of the intestinal form, is one point of difference.

Again, in the mouth form, the undulating membrane is no stouter than the anterior flagella, and does not terminate in a free posterior flagellum, nor is the basal chromatinic rod connected with it well developed, whereas, in the intestinal form the undulating membrane is stouter than the anterior flagella, and there is a posterior flagellum and a well-developed basal rod.

The nucleus also and the axostyle, in the two forms, present further points of difference. In the mouth form, the nucleus seems to stain rather diffusely, and distinct chromatin granules are very rarely discernible, whereas, in the figures of the intestinal form chromatin granules are shown. The axostyle in the mouth form stains rather darker than the cytoplasm whilst in the intestinal form it appears as a clear refractile bar, according to Wenyon (1915).

Unfortunately, it has not been found possible to make a careful comparison of the mouth form described above with good examples of the intestinal form. The latter was obtained in faeces smears on one occasion, but the majority of the organisms present were in a degenerate condition; some of them showing in life the production of small lateral pseudopodia, characteristic of the dying condition. The few that were

found to present a normal appearance on the stained films agreed well with the illustrations of the intestinal form, as figured by Brumpt (1913).

Judging then by the aid of the published figures of the intestinal form it is in the writer's opinion preferable to separate this from the mouth form, and not unite them under the specific names of *hominis*, as done by Doflein, or *intestinalis* by Jollos.

The names *hominis* or *intestinalis* should be employed for the intestinal form alone (the former has the priority claim) whilst the writer proposes the specific name *buccalis* for the mouth form, at any rate provisionally until such time as further research can prove that the important morphological differences presented by the two forms are merely due to different environmental conditions.

The fact that the mouth form described in this note, possesses four anterior flagella, does not affect the question of specific difference; it merely shows that it should be placed in the subgenus *Tetratrichomonas* created by Parisi (1910). Tetratrichomonad and even pentatrichomonad forms occur in the intestines according to Wenyon (1915), though very rarely.

The name therefore proposed for the flagellate in question is *Tetratrichomonas buccalis*, n. sp.

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EXPLANATION OF PLATES XX–XXII

PLATE XX.

Photomicrographs, all magnified 1325 diameters, except Fig. 7, which is magnified 820 diameters.

Fig. 1. *Entamoeba gingivalis*, trophic form showing characteristic ingesta; the nucleus is seen on the left side of the amoeba as a ring of chromatin with a central granule. Stained. Iron-haematoxylin.

Fig. 2. *E. gingivalis* form stained with safranin and licht-grün, and showing the feebly stained nucleus as a ring with central karyosome. The nucleus on the right side just overlies a more deeply stained food body which is slightly out of focus.

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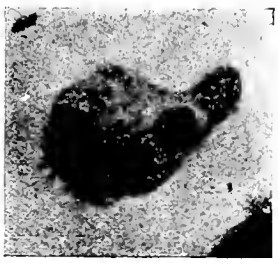

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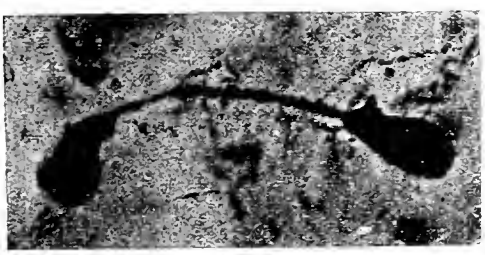

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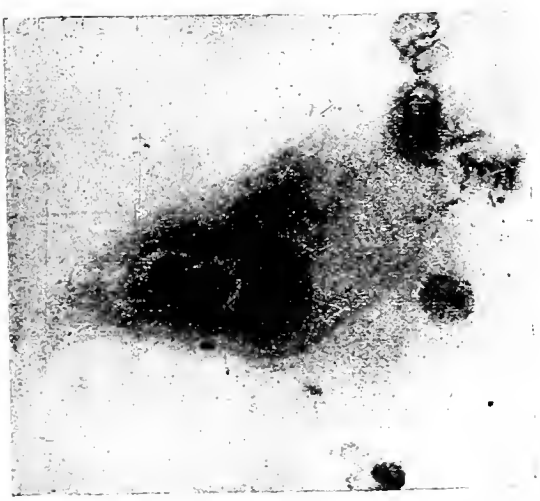

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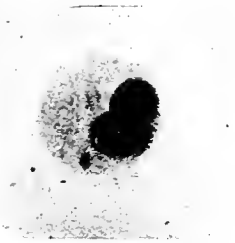

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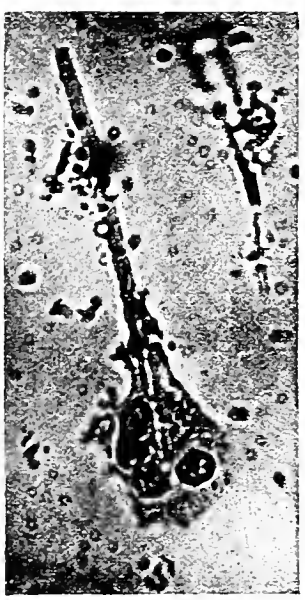

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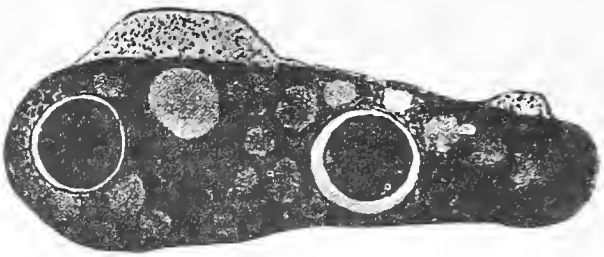

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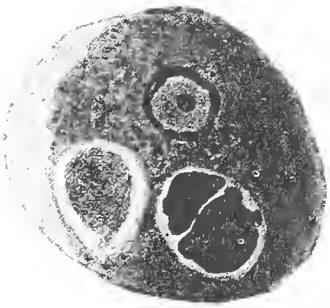

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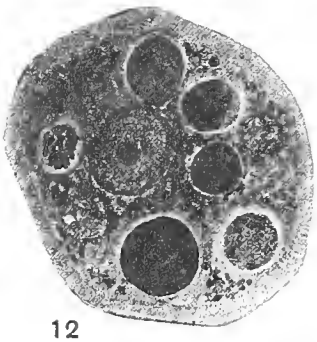

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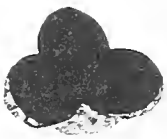

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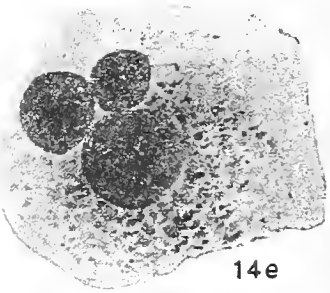

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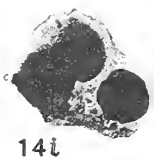

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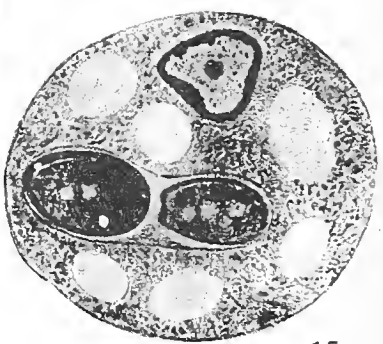

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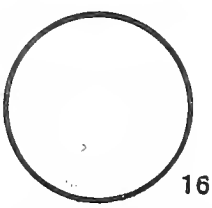

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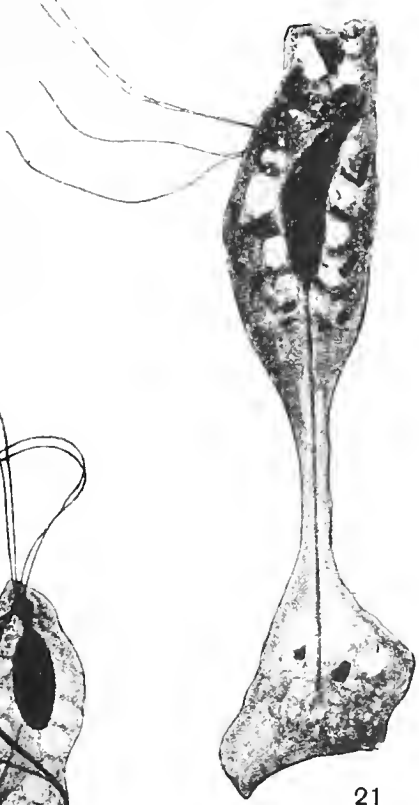

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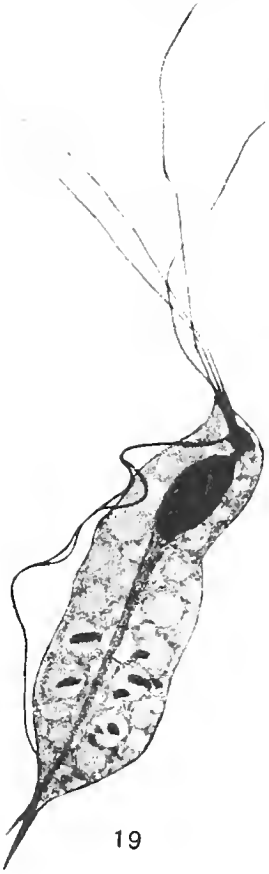

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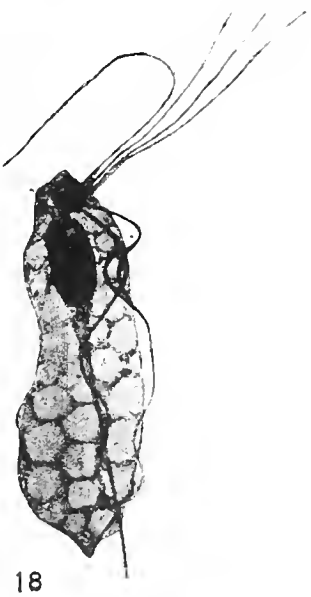

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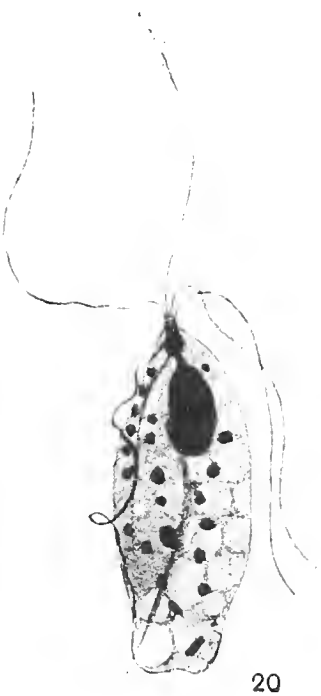

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Fig. 3. *E. gingivalis* forms showing *bacteria* ingested as the source of food. Stained. Iron-haematoxylin.

Fig. 4. *E. gingivalis*. trophic form showing characteristic round bodies within food vacuoles, and rods of bacteria at posterior end. Iron-haematoxylin.

Fig. 5. *E. gingivalis*, very small form 7·5μ long, from pyorrhoea material from child of 5 years. Iron-haematoxylin.

Fig. 6. *E. gingivalis*, bi-nucleate form with the nuclei in focus, the cytoplasm is out of focus. Iron-haematoxylin.

Fig. 7. *E. gingivalis*, late stage of fission, the connecting strand of protoplasm is slightly out of focus. Iron-haematoxylin.

Fig. 8. Endothelial cell with an ingested polymorphonuclear leucocyte towards lower end slightly overlapping the cell nucleus. Iron-haematoxylin. From pyorrhoea pus.

Fig. 9. Polymorphonuclear leucocyte from gum, ingesting a rod of bacteria and showing the open character of the lobed nucleus. Fixed absolute alcohol, stained methyl-green.

Fig. 9 *a*. Polymorphonuclear salivary corpuscle showing the rounded almost homogeneous lobes of the nucleus. Stained iron-haematoxylin.

PLATE XXI.

Camera lucida drawings, all magnified 2725 diameters. All stained with iron-haematoxylin.

Figs. 10, 11, 12, 13, drawings of *E. gingivalis* showing characteristic inclusions of various shapes and sizes and staining reaction. Fig. 13 shows the cytoplasm of the amoeba in an alveolate condition, whilst the inclusions show small pieces of cytoplasm attached to the chromatinic material. Fig. 13 *a*, *b* and *c*, deeply stained nuclear lobes with attached cytoplasmic fragments from same film as Fig. 13.

Fig. 14 *a–m*, disintegrating salivary corpuscles and their products, showing the same characteristic appearances as the amoeba inclusions, drawn from salivary and tonsillar smears and from pyorrhoea pus and food *débris* smears.

Fig. 15. *Entamoeba coli*, a small trophic form showing two ingested yeasts lying in one vacuole, note the different appearance of these from the inclusions in *E. gingivalis*.

Fig. 16. Outline of red blood corpuscle drawn to the same scale of magnification for purposes of comparison.

PLATE XXII.

Tetratrichomonas buccalis n. sp. Camera lucida drawings magnified 4100 diameters. All stained with iron-haematoxylin.

Figs. 17–20. Four different free swimming trophic forms showing the four anterior flagella, the undulating membrane and the relations of nucleus, blepharoplast and rhizoplast, and also the axostyle. In Fig. 19, the chromatinic basal rod connected with the undulating membrane appears to be absent.

Fig. 21. An organism in a much extended amoeboid condition showing numerous ingested bacteria and the axostyle as a long narrow rod. The outline of the nucleus is lost, owing to the differentiation having been carried rather too far.

SOME CESTODES FROM JAPANESE SELACHIANS.

INCLUDING FIVE NEW SPECIES.

By Prof. SADAO YOSHIDA.
(*Pathological Department of Osaka Medical Academy.*)

(With Plate XXIII and 4 Text-figs.)

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Rhynchobothrium laciniatum n.sp.	588

Several years ago I collected cestodes parasitic in Selachians found in Japanese waters. Most of them were mature and well preserved. The specimens are referable to ten species, of which five belong to known species and five are new to science. Since the published descriptions of the known species are not entirely in accord with my observations, and some of the species are not satisfactorily described anatomically, I shall re-describe the known forms briefly in the following pages.

1. **Phyllobothrium lactuca** van Beneden, 1850.

Numerous specimens were obtained 10. v. 1911, from the spiral valve of *Cynias manazo* (Bleeker) caught near Hiroshima. They were found associated with some other cestodes, one of which I describe as a new species.

External character. Length 60—80 mm. Head subglobular,

2—3 × 2·5—4 mm. Bothridial border strongly folded appearing like a cauliflower. The head agrees well both in size and in shape with the figure given by van Beneden (1850). Neck tolerably long, broadening posteriorly; 10—20 × 0·5—1·1 mm.; the surface bears numerous transverse wrinkles which give rise to an appearance of segmentation.

Strobila short, thick, oval in cross section. The breadth of segments increases gradually towards the posterior end, reaching a maximum near the posterior extremity where it measures 2·7—5·0 mm.; thence it gradually tapers caudally. The length of the segments also gradually increases posteriorly, reaching a maximum in the last segment which is 0·8—1·5 mm. long. The dimensions of various parts of body are as follows (in mm.):

	Head	Neck	First segment	Middle segment	Maximum segment	Last segment	Total
Length	2—3	10—20	very short	0·3—0·5	0·3—0·5	0·8—1·5	60—80
Breadth	2·5—4·0	0·5—1·1	1·5—2·0	2·7—4·5	2·7—5·0	1·5—2·0	

The worm is very thick, especially in mature segments in which the uterus is distended by eggs. The posterior third or fourth of the strobila is atrophied, as the result of the discharge of ova. A series of ruptures, for the discharging of ova, take place in the median ventral line of mature segments. In some specimens the openings enlarge and become continuous with those of the adjacent segments thus forming a shallow groove on the ventral median line. This is the most remarkable external feature of my specimens.

Genital pores lateral, situated in the middle of the segment. Cirrus and distal part of cirrus pouch are often protruded from the genital opening; this is readily seen by the naked eye.

Genital organs. Testes oval or spherical in shape, scattered throughout the medullary field excepting the posterior portion where the female organs are situated. Cirrus pouch thin-walled, elongate pyriform in shape, proximally nearly attaining the middle of the segment; opens into the bottom of the distended genital cloaca which is lined with the ciliated cuticular layer. Distal end of pouch sometimes evaginated. Cirrus unarmed, long and slender.

Vas deferens coiled in the cirrus-pouch; its wall very thick, with inner circular and outer longitudinal muscle bundles, and lined with a ciliated cuticular layer, as in the genital cloaca. Vas deferens on leaving the cirrus pouch is thin-walled and strongly coiled. In sections of mature segments the loops of the vas deferens are often pushed out when the eggs are discharged through rupture of the segment.

Vagina: opens into the bottom of the genital cloaca antero-ventrally to the cirrus opening and runs straight in along the cirrus pouch, passing ventrally to both the lateral nerve cord and dorsal canal of the excretory system; its inner end reaches the median line of the segment and becomes continuous with the oviduct. The vaginal wall thick, with structure similar to vas deferens.

Ovary: consists of four lobes, lying at the dorsal and ventral margins of both halves of the medullary field, being united medially by narrow processes, forming an isthmus. The oviduct arises in the middle of the isthmus, runs ventrally, then posteriorly in a slightly winding course to unite with the inner end of vagina. The united canal runs dorsally to meet the shell gland, which is situated postero-dorsally to the isthmus.

Uterus: lies ventrally and longitudinally in the median line; in gravid segments it occupies a large portion of the medullary field. The connection of uterus and shell gland is not well seen in my sections probably owing to faulty preservation.

Yolk gland: strongly developed, situated in both the lateral extremities of the medullary field. It lies at the margin of the medullary field, and extends into it, occupying the space between the lateral nerve and the region occupied by the excretory canals. Each lateral end of the ovarian lobe comes into contact with the inner end of the yolk gland.

Musculature: the longitudinal muscles are well developed; they occupy nearly all the cortical field; the muscular bundles are numerous and arranged radially.

Affinities. I am inclined to identify this worm with *Phyllobothrium lactuca* van Beneden, although there are some slight differences between my description and that of the author of the species. The chief differences are (1) the smallness of the strobila, and (2) the shortness of the gravid segments. *P. lactuca* is stated to measure 150—350 mm. or more in length and 2—5 mm. in breadth; it is much larger than my specimens, whose segments are always broader than long throughout the whole length of the body. But the last segments of *P. lactuca* are said to be longer than broad. Van Beneden described and figured a very long segment which is 12—15 × 4—5 mm. My specimens are shorter than this in total length (including the last gravid segments), but these differences may be due to post-mortem changes. These slight differences, I think, do not suffice to separate my form from *P. lactuca*.

Specimens resembling the above were collected 29. v. 1907, from *Cynias manazo* in Tokyo. Though much narrower they were nearly as long as specimens obtained in Hiroshima; the head is smaller, the border of the bothridia thinner and more loosely folded; the strobila is smooth, without rupture or groove.

2. **Crossobothrium angustum** (Linton, 1889).

(Plate XXIII, figs. 1—5.)

Syn. *Orygmatobothrium angustum* Linton, 1889.

The material at my disposal was collected by Mr T. Tsuchida from the spiral valve of *Triakis scyllium* Müller and Henle, at Misaki 11. viii. 1906. The characters of this worm agree well in most respects with those of Linton's species and it is reasonable to suppose that they are identical. Linton's statements, made on the several occasions, are based chiefly upon the external characters and not on the internal structures. Therefore, my description which follows is more complete as it includes also a consideration of the internal structures.

External features. The worm measures 10—30 mm. in length; most specimens attain a length of 25—30 mm. In large specimens the widest posterior segment measures 1·1 mm. in breadth, whilst the narrowest portion at the neck measures 0·1 mm. in breadth. The head (Pl. VII, fig. 1) is generally pyramidal in shape and the size varies slightly according to the state of contraction of the bothridia, being on an average 0·6 mm. × 0·6 mm. at the posterior widest portion. There are four bothridia, unarmed and elongated oval in shape when at rest; anteriorly narrowed, somewhat roundly pointed, surmounted at the apex by a supplemental disk or accessory sucker (axiliary acetabulum); posteriorly rounded, broader than anteriorly, and flaring away from the neck so as to turn its surface outward. The margin of the bothridium is entire and is more or less thickened. The bothridium is 0·57—0·6 mm. long and 0·3—0·35 mm. broad at the widest part near the posterior extremity. Linton observed in living, actively moving specimens, that the anterior end of the bothridia "frequently elongated and curve outward and back in horn-like prolongation. An opposite movement is that in which the anterior ends of bothridia are closely oppressed and the broadly rounded posterior end are curved outward and forward." Unfortunately I have not had any opportunity to observe living specimens, and in my alcoholic specimens I do not find the prolongation of the anterior ends of bothridia. The

neck (Pl. XXIII, fig. 1) is narrow and long, measuring 0·5 × 0·17 mm. anteriorly where narrowest; it is marked with closely set transverse rings, giving its edge a serrated outline.

The strobila is tenuous in the anterior part of body and gradually widens posteriorly, reaching its maximum breadth (about 1·1 mm.) at the posterior end (Pl. XXIII, figs. 2 and 3). The segments rapidly increase in length toward the caudal end, the anterior segment measures 0·12 × 0·12 mm.; the ripe posterior segment attains a length of 3—4 mm., being three or four times longer than broad. The worm's surface is marked by closely set, transverse, slightly notched rings which give its margin a serrated appearance. This appearance, as noticed by Linton, can readily be seen with a low power, and constitutes a good specific character. The genital apertures are situated irregularly and alternately, opening at about the middle of the anterior half of the segment.

Musculature: there are two sets of longitudinal and transverse musculatures, situated as in other cestodes; both sets are weakly developed.

Male organs. The testes (Pl. XXIII, figs. 4 and 5, *H*) occupy the anterior two-thirds or three-fourths of the medullary field, they are oval in shape, measuring about 0·09—0·15 × 0·05—0·055 mm. Anteriorly in each segment, the testes spread all through the medullary field, but towards the middle and the posterior segments they are displaced dorsally by the female organs. The cirrus pouch (Pl. XXIII, figs. 4 and 5, *B*) opening into the common genital cloaca, is situated at right angles to body margin; it is oblong in shape, and measures 0·36—0·43 × 0·17—0·25 mm.; in mature segments the pouch is usually distended by the seminal vesicle enormously enlarged with the spermatozoa, and assumes an unusual form; the pouch wall is very thin and surrounded by a single cell-layer.

The vas deferens (Pl. XXIII, figs. 4 and 6, *L*) in the cirrus pouch is strongly convoluted. In young segments it is of uniform structure throughout its whole length, its wall being surrounded by a layer of cells with distinct nuclei and its diameter being nearly uniform (0·03 mm.) excepting distally, where it enlarges to form the pyriform duct (0·07—0·10 × 0·045—0·05 mm.). In mature segments the vas deferens in the cirrus pouch exhibits two easily distinguished parts; (*a*) the distal part which is thick-walled, lined with minute retrograde spinose projections and surrounded by a thick layer of cells; (*b*) the proximal part which is thin-walled, its lumen being enormously distended with

the spermatozoa for it functions as a seminal vesicle. On leaving the cirrus pouch the vas deferens is coiled many times basally to the pouch; its further course to the testes has not been traced in my preparations; the vas deferens is surrounded throughout by a cell-layer.

FEMALE ORGANS. The essential parts are situated posteriorly in the segment. The vagina (Pl. XXIII, fig. 5, *V*) beginning at the common genital cloaca, runs towards the median line along the anterior edge of the cirrus pouch, then bending nearly at right angles it runs back to in front of the shell gland where it enlarges and assumes an irregular shape. The narrow proximal part of vagina passes by the shell gland posteriorly and turns again anteriorly to open into the shell gland. Where it bends, it unites with the oviduct. The vaginal wall is of uniform thickness, and is surrounded by a cell-layer with distinct nuclei; its structure is best seen in sections. It is nearly uniform in diameter (about 0·02 mm.) throughout its length, but for the dilated portion lying anterior to the shell gland; the dilated portion may serve as a receptaculum seminis.

The shell gland (Pl. XXIII, fig. 5, *sd*) is spherical, with margins irregularly lobed, and measuring 0·064 mm. It is situated in the median part of the posterior region. Anteriorly, its canal runs forward along the vagina to near the middle of the segment and opens into the uterus, which lies longitudinally in the median field. The canal measures about 0·009 mm. in width and is surrounded by thick masses of cells.

The uterus (Pl. XXIII, fig. 5, *U*), in young segments, appears as a mere longitudinal cell rod, containing a canal which gradually grows larger toward the posterior segments. In the mature segments the uterus is much distended with the ova, and ultimately it completely fills the available space in the segment.

Running forward from the side of the shell gland is the yolk duct (Pl. XXIII, fig. 5, *G*) which forks anteriorly to form two canals running laterally to the yolk glands on both sides of the segment. The yolk glands are situated in the lateral areas, extending dorso-ventrally inside the transverse muscle bundles, which separate the medullary field from the cortical; they occupy the anterior two-thirds or three-fourths of the segment; their arrangement is best seen in sections (Pl. XXIII, fig. 4, *D*).

The ovary (Pl. XXIII, fig. 4, *K*) is situated posteriorly in the segment, its antero-lateral margins being continuous with the posterior end of yolk glands; it is arranged in four groups or laminae, two on each side. The lateral groups are placed on the dorsal and ventral side of medullary

field respectively and the inner ends are continuous with one another before the shell gland, forming a small isthmus. In cross sections through the level of an isthmus, the arrangement of ovarian laminae resembles an H, with the four limbs thickened, the horizontal part being very short or entirely obliterated. Each lamina, moreover, is indented superficially and irregularly into a number of rounded lobes.

The oviduct arises at the middle part of the isthmus, where it forms the "egg-swallowing apparatus"[1] of German authors; thence it runs backward along the vagina to unite with it at some distance from the shell gland. The common duct leads forward into the shell gland. The structure of the oviduct is similar to that of the vagina.

It is not an easy task to find the complicated connections of various parts of the female organs. They are diagrammatically shown in Pl. XXIII, fig. 5.

3. **Orygmatobothrium velamentum** n.sp.

(Pl. XXIII, figs. 6—11.)

The material was obtained from the spiral valve of *Cynias manazo* (Bleeker) on 10. v. 1911, in Hiroshima. The specimens were numerous and associated with other Cestodes such as *P. lactuca* (already referred to). Most of them are fully grown and mature, but I have not found gravid segments in any specimens.

External characters. The mature form measures 30—40 mm., besides which there are some smaller young-stage specimens. The head (Pl. XXIII, fig. 6) is provided with four cup-like bothridia, disposed crosswise and provided either with very short peduncles or nearly sessile. The bothridia are directed forward and slightly outward, their anterior subcircular surfaces being nearly at right angles to the long axis of the head; each bothridium bears two small accessory suckers of nearly the same diameter, one at the anterior angle, the other central; the anterior sucker is more easily visible than the central one, but by the contraction of the anterior portion of the bothridium, it is often hard to detect; the central sucker is weakly developed and at times difficult to see. This obliteration of the accessory suckers is frequent, and when I first studied the Cestode I falsely concluded that they were absent and that I was dealing with a member of the genus *Anthobothrium,* but the subsequent examination of many good specimens revealed my error. The border of the central accessory

[1] Hereafter called "egg-swallower."

sucker protrudes slightly from the bottom of the bothridium, whose border, in turn, is thickened, entire, and not folded. The bothridium contour is only broken at the anterior sucker, and its thickened margin is surrounded by a second thin membranous fold or velum, which constitutes a noteworthy character peculiar to this species. The head measures 1·6 mm. and each bothridium 0·5—0·6 mm. in diameter.

The strobila is slender and delicate in the anterior third or fourth; it is widest and nearly uniform in breadth at about the middle third and gradually narrows in the last third of its length; anteriorly it is wrinkled irregularly (Pl. XXIII, fig. 6). In some wrinkles—they show irregularity in structure—the posterior border is entire while in others it is slightly lobed. Over about a third of the anterior portion of the body length, the wrinkles gradually grow farther apart (0·07—0·08 mm.), but they soon diminish in length. Over about two-thirds or three-fourths of the body length there are slight cuticular wrinkles, which give a serrated, pseudo-segmented, appearance to the margin.

The slender neck gradually broadens posteriorly; it measures 0·03—0·36 mm. anteriorly and 0·7—0·8 mm. posteriorly.

Anteriorly the segmentation is not visible externally but it is indicated internally by the cell masses of the genital Anlagen. Segmentation begins at a distance of 8—10 mm. from the head. The segments which follow rapidly increase in width, attaining a maximum breadth of 1·1 mm. at a point two-fifths or one-third along the body length. These broadest segments succeed each other for some distance after which the segments gradually taper toward the posterior end (Pl. XXIII, figs. 7 and 8). The length of segments increases toward the posterior end of body, hence they vary in form from the wide rectangles in front to squares in the middle of the body and elongated segments behind. The anterior segments measure 0·2 × 0·8—0·9 mm., the widest 0·8 × 1·1 mm., whilst the posterior segments are 1·5—2·5 mm. in length and oval in cross-section (Pl. XXIII, figs. 8 and 9).

I possess many other specimens than those above described which are slightly thicker and have shorter necks and posterior segments.

The genital openings are irregularly alternate, lateral, situated a little anterior to the middle or at the anterior third of the segment. The genital organs are only fully developed in a few of the posterior segments, but even here the eggs are not massed in the uterus.

MALE ORGANS. The testes (Pl. XXIII, figs. 8, 9 and 11, *H*) fill the space between the other genital organs in the medullary field; they are absent posteriorly in the segment; which is mainly occupied by the

female organs; each testis is oval in shape, measuring 0·06—0·07 × 0·04—0·05 mm.

The vas deferens (Pl. XXIII, fig. 11, *L*) is much coiled anteriorly to the cirrus pouch, the pouch being situated at about the anterior third of the segment. The duct is thin-walled and 0·01—0·023 mm. in diameter; it enters the cirrus pouch, in which it is coiled many times and enlarges its diameter forming the cirrus distally (Pl. XXIII, fig. 11). In the cirrus pouch the duct has a thick wall consisting of two muscular layers, the inner circular and the outer longitudinal. There are about 50 longitudinal bundles of muscle fibres. The duct wall is lined with chitinous spinules and surrounded by distinctly nucleate cellular masses.

The cirrus pouch (Pl. XXIII, figs. 8 and 11, *B*) is very large, extending from its opening to the other side of the medullary field, it is ovoid in shape and its wall is very thin; it opens into the common very thick-walled genital cloaca, which measures 0·17 × 0·08 mm.

Female organs. These occupy mainly the posterior part of the segment, the vagina and uterus, however, run through the segment longitudinally. The ovary (Pl. XXIII, figs. 8, 9, 10 and 11, *K*) is situated posteriorly in the segment, it forms four irregular groups of elements extending dorsally and ventrally. Externally each group is continuous with the yolk gland and internally it protrudes into the segment, where the ovarian groups unite midway to form an isthmus (figs. 9 and 10, *I*); the latter communicates with the oviduct by means of the "egg-swallower" (figs. 9 and 10 *E*) which is situated in the middle of the isthmus, its wall being very muscular, it measures 0·04 mm. in diameter.

The oviduct (Pl. XXIII, figs. 10 and 11, *O*) runs ventrally from the "egg-swallower" and soon unites with the proximal end of the vagina coming from the antero-dorsal side, the common duct then running dorsally to open into the shell gland (Pl. XXIII, figs. 10 and 11, *SD*). The oviduct wall and proximal part of the vagina possess the same structure, the muscular wall being lined with cuticular cilia or spinules.

The yolk gland (Pl. XXIII, figs. 8, 9 and 11, *D*) forms four longitudinal columns running forward from the posterior end of segment dorsally and ventrally to the lateral nerve cords, and lying between the marginal and medullary fields. Each column is of elongated oval shape in cross section (Pl. XXIII, fig. 9, *D*). The shell gland (Pl. XXIII, figs. 10 and 11, *SD*) is spherical or ovoid in shape and is situated a little postero-

dorsally to the isthmus. The gland receives the oviduct and yolk duct posteriorly; the other duct arises anteriorly, it is slightly coiled and runs dorsally to the vagina, and, about midway along the segment, it bends to open into the uterus, which is situated ventrally along the median line (Pl. XXIII, figs. 10 and 11, *U*). In my specimens the uterus consists of a mere cell mass with a small central lumen not containing ova even in the posterior segments.

The vagina (Pl. XXIII, figs. 10 and 11, *V*) opens into the common genital cloaca dorsally to the cirrus opening; thence it runs inward in front of the cirrus pouch, bends backward, and upon attaining the ovarian region, it narrows and runs a winding course and unites with the oviduct. The vagina measures 0·04 mm. in width distally and 0·02 mm. in width proximally; it is widest a little in front of the isthmus. The vaginal wall is tolerably thick and is lined with a layer of cuticular spinules.

Affinities. This worm bears some resemblance to *C. laciniatum* Linton, especially in the shape of scolex and in the laciniated border to the segments, it differs, however, chiefly as follows:

The presence of (1) the velum about the bothridial rim, and (2) of a central accessory sucker upon each bothridium; (3) the mode of attachment of the bothridium in respect to the axis of the head; (4) difference in form of laciniated border at the different parts of the strobila; (5) difference in the shape of the last segment (in adult *C. laciniatum* the length and breadth are nearly equal, whereas in my specimens the length is much greater than the breadth); (6) the total length (110—212 mm.) of *C. laciniatum* is much greater than in my specimens.

Diagnosis. Length 30—40 mm. or a little more. Head provided with four cup-like bothridia, measuring 1·6 mm. in diameter. Bothridium sessile or with short peduncle, turning forward and slightly outward, margin entire, thickened, surrounded by second membranous fold or velum; each bothridium measuring 0·5—0·6 mm. in diameter, provided with two accessory suckers, one on the anterior corner and the other central upon the bothridium; central accessory sucker weakly developed, appears as a mere depression or groove on the bottom of the bothridium.

Strobila slender and delicate in the anterior third or fourth, widest and nearly uniform in breadth in the middle third or more, and a little narrower in the posterior third of body length. Cuticular wrinkles on the body are somewhat larger in the anterior, but very minute in the middle and the posterior part of strobila. Neck long, slender,

measuring about 8 × 0·30—0·36 mm. anteriorly where narrowest and 0·7—0·8 mm. posteriorly. The segments broaden and lengthen posteriorly for some distance, then slightly decrease in breadth, the last segments being much longer than broad.

Genital openings irregularly alternate, situated a little anterior to the middle of lateral margin or at a distance of one-third of a segment-length from anterior border. Testes distributed throughout the space among the other genital organs; oval in shape, measuring 0·06—0·07 × 0·04—0·05 mm. Vas deferens with thin wall; much coiled near the anterior side of cirrus pouch; measuring 0·01—0·023 in diameter, much coiled in the cirrus pouch, its muscular wall thickened and lined by a layer of cuticular spinules and surrounded by well nucleated cell masses. Cirrus opens into the common genital cloaca. Cirrus pouch with thin wall, large, oval in shape extending to near aporose side of medullary field.

Ovary situated near the posterior end of segment, consisting of four irregular lobes, each lying on dorsal and ventral sides of segment. Inner ends of ovary converge toward the median line of segment where they unite to form an isthmus of ovarian groups. Yolk glands consist of four longitudinal columns running antero-posteriorly dorsally and ventrally to the lateral nerve cords on both sides; they lie between the marginal and medullary fields; each column elongate oval in cross section. Shell gland spherical or oval and situated a little postero-dorsal to the isthmus. Uterus running longitudinally along ventro-median line of each segment; connected with shell gland by a small duct running from shell gland anteriorly on dorso-median line and opening into uterus about midway along segment. Vagina begins with common genital cloaca at dorsal side of cirrus opening, runs inward along the anterior side of cirrus pouch, bends posteriorly near the inner end of pouch and passes back to the ovarian region where it rapidly narrows, and, pursuing a winding course, unites with the oviduct. United canal runs dorsally to open into shell gland. Wall of vagina and oviduct of similar structure being composed of thick muscular layers and lined with the cuticular spinules

Host. *Cynias manazo* (Bleeker), spiral valve.

4. **Acanthobothrium coronatum** (Rud., 1819) van Beneden, 1849.

Syn. *Bothriocephalus coronatus* Rudolphi, 1819.
B. bifurcatus Leuckart, 1819.
Tetrabothrius coronatum (Rud., 1819) Wagener, 1854.
Calliobothrium coronatum (Rud., 1819) Diesing, 1863.
C. corollatum (Abildg., 1790) Mont., 1887 of Beauchamp, 1905.

The specimens were obtained from the spiral valve of *Dasyatis akaei* (Müller and Henle) on 5. iv. 1913, in Nakatsu, West Japan. They are very abundant and almost all of equal length (200 mm.). This is apparently identical with the well-known species *A. coronatum* (Rud., 1819) van Beneden, but my specimens differ somewhat from the descriptions thereof by the several authors. I shall describe these differences and my observations on this worm.

External characters. Dimensions of various parts of worm, fixed by formalin, are as follows (in mm.):

	Head	Hook	Neck	1st segment	Post. segment	Last segment
Length	1·0	0·18—0·19	80—100	0·1	0·3	0·5—0·6
Breadth	1·2	0·03	0·46—0·6	0·75	1·3—1·5	1·3—1·5

The scolex or head is subquadrate, broadening slightly behind. The bothridia are four in number, opposite one another, and of characteristic elongated oval form; each bothridium is divided into three unequal loculi by two transverse costae, the anterior loculus is the largest and deep, the posterior one the smallest and shallow; the bothridium is provided with a pair of bifurcated chitinous hooks at the anterior corner of the first loculus. The hook measures 0·18—0·19 mm. in length from base of the common stalk to the tip of prong; the basal common stalk is shorter than any of the prongs; the inner prong is a little shorter than the outer one; the base of the prong measures 0·03 mm. in diameter. In front of each pair of hooks there is the single accessory sucker, which is more or less muscular and deep.

The neck is widest just behind the head, being nearly as wide as the posterior part of the head; it gradually narrows toward the middle, where it is narrowest, then widens gradually toward the first segment. In my specimens the neck is fairly long, as shown in the above table; this may be due to their preservation in an extended state. All previous authors describe the neck as short. The neck merges insensibly into the strobila, the anterior segmentation being very obscure

and appearing as mere transverse striation, but the segments gradually increase in length and breadth. The last widest segments measure 1·3—1·5 × 0·5—0·6 mm. Thus the segments are broader than long throughout the whole length of the body; they number about 450. The genital openings are irregularly alternate.

Genital organs. All my specimens are immature and there is no trace of the genital gland excepting the testes. The testes and the genital ducts appear in the posterior segments only and the latter are not as yet differentiated into the male and female ducts, being either mere cell masses or small canaliculated groups of cells. The testes scatter in the medullary portion of segment, being arranged in a single layer. In the anterior section of the segment, they occur in one row, 14 in number being traversed between two lateral excretory canals; in the posterior part of the segment, the transverse row of testes is separated into two lateral parts by the Anlagen of the genital ducts; on the porose side the row consists of 6—10 testes and on the aporose side 8—12. The testis is oval or spherical in shape, measuring 0·03—0·04 mm. in diameter. The genital ducts are represented at this stage by undifferentiated masses of cells which pass between the dorsal and ventral excretory canals.

Musculature. The longitudinal bundles of muscles are strongly developed, especially in the neck region where they are clearly visible from the exterior in total-preparations. The number of bundles increases in the posterior part of worm there being ca. 110 in all. The muscular bundle is largest along the median line of the segment both ventrally and dorsally, and decreases in size laterally.

Excretory canals. There are four main canals as in other Cestodes; both the dorsal and ventral canals are nearly equal in width anteriorly, measuring 0·022—0·03 mm.; seen in cross section they are round but in the posterior region they become oblong in cross section; the dorsal canal is much smaller (0·22 × 0·01 mm.) than the ventral (0·034 mm. × 0·02 mm.).

Affinities. From the above description it is clear that the worm most resembles *A. coronatum* (Rud.), therefore I provisionally identify it with this species, though there are some points of difference: (1) longer neck, (2) shorter posterior segments due probably to the immature state of my specimens, (3) larger size.

5. **Acanthobothrium ijimai** n.sp.

(Pl. XXIII, figs. 12—13 and Text-figure 1.)

This cestode was collected by Prof. Dr Ijima from the spiral valve of *Dasyatis akaei* (Müller and Henle) in Tokyo, in Feb. 1886. Only three specimens were well preserved in alcohol.

External characters. The specimens are all of nearly equal length, about 40 mm. The head (Pl. XXIII, fig. 13) is provided with four bothridia arranged diagonally and directed forward, reminding one of a four-leaved clover viewed frontally; the bothridia are sessile and closely united together for some distance as shown in the figure cited, each bothridium is ovoid in shape, measuring 1·6—1·7 × 1·2—1·4 mm. The sucking surface of the bothridium is divided into three unequal loculi by two transverse septa, the anterior loculus is the largest and the posterior the smallest; the bothridial border is entire and fairly thick, the septa well developed.

Anterior to the anterior loculus the bothridium is provided with one pair of bifurcated hooks, which look like the letter (r) under a low magnification. The hook is very small, measuring 0·09—0·11 mm. in total length. There are three accessory suckers one large (0·1 mm.), and two small (ca. 0·05 mm.) situated in the anterior pad of each bothridium, and disposed as shown in the figures; two smaller accessory suckers, not readily visible, can only be detected by careful examination. The neck varies in length between 0·2 and 0·6 mm. according to the degree of contraction; it forms the narrowest part of the body.

Posteriorly the segments gradually increase in length and breadth, the maximum breadth being reached near the posterior end whilst they taper posteriorly further back. Segments of maximum size measure 0·4—0·6 × 1·1—1·5 mm.; their length is greatest in the last segments or segment; here they are longer than broad in one specimen, and slightly broader than long in two others. The genital pores are lateral and irregularly alternate.

Genital organs. All three specimens are immature and the genital organs have not as yet been fully developed except the testes. The testes are spherical or oval in shape and scattered throughout the medullary field excepting the space occupied by the female organs. Most of the vas deferens is in the progressive stage of development, its distal part with the cirrus pouch has not yet become differentiated from the cellular mass of the Anlage, which shows a longitudinal central

slit. The vas deferens and vagina pass between the dorsal and ventral longitudinal excretory canals and ventral to the nerve cord.

The female organs are all still young and are presented by cellular masses. The ovarian Anlage is situated posteriorly in the segment and consists of the right and left lobes united in the middle.

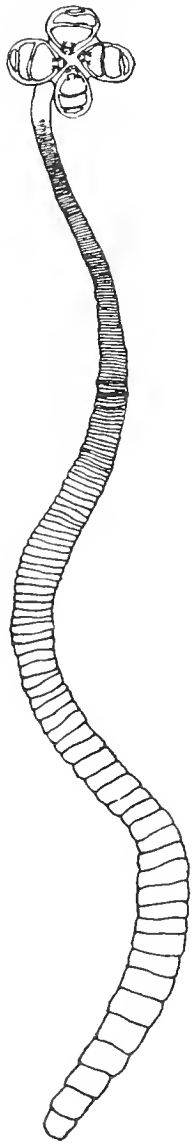


Fig. 1. *A. ijimai* n. sp. × 3.

The nerve cord is 0·033 mm. in diameter, and runs longitudinally between the medullary and cortical fields.

Musculature. The longitudinal muscles consist of two sets, the one running just beneath the surface (dermal muscle), the other between the marginal and median fields. The dermal muscles consist of about 150—160 bundles of fibres which are smaller than those of the inner set. The latter set consists of about 40—50 bundles, each measuring 0·02—0·04 mm. in diameter. The bundles gradually decrease in size laterally the median portion being the largest.

Affinities. This worm cannot be referred to a known species. It shows a slight resemblance to *Calliobothrium farmeri* Southwell in the shape of the head. Southwell writes: not only are the bothridia "sessile, but they are united together for some distance along their length in such a way that the anterior view of the head is almost that of a square. Each bothridium extends to the centre of the head, becoming produced or lumpy anteriorly, so that all four remain distinct. Between the centre of head and each pair of hooks there is a minute accessory papilliform sucker...." In these respects my specimens differ from Southwell's, that is, all the bothridia are so closely aggregated that there is no space between any two adjacent bothridia, and only a small area is found at the apex of head between their anterior tips. The accessory suckers of each bothridium are three in number in my species, while there is only one sucker in *C. farmeri*. The hooks of *C. farmeri* are described and figured by the author (fig. 3 *a* Pl. V) as simple, though in his fig. 3 *b*, Pl. V, they are slightly bifurcated.

If the features of head : bothridia and hooks, length of worm,

and form of segments, etc., are taken together they are sufficient to establish the species as new to science. I accordingly name it *Acanthobothrium ijimai* in honour of the collector of the specimens.

Diagnosis. Length 40 mm. or more (probably longer than this in fully matured form). Head considerably large, provided with 4 sessile bothridia, which are arranged in a diagonal position. All bothridia turn forward and are united together closely by their anterior half, giving a square appearance when seen frontally. The square is placed nearly at right-angles to the long axis of the head. Each bothridium is oval in shape, it measures 1·6—1·7 × 1·2—1·4 mm. and is divided into three unequal loculi by two transverse septa, the anterior the largest and the posterior the smallest. One pair of bifurcated hooks is planted on the proximal border of the anterior loculus of each bothridium. The hook is small in size and r-shaped. Three accessory suckers occur on the proximal end of each bothridium, one is large and occupies an antero-median position, two are smaller and situated postero-laterally to the first.

Neck varies in length between 0·2 and 0·6 mm. according to the condition of contraction. The segments gradually increase in breadth, reaching a maximum (1·1—1·5 mm. or more) and tapering toward the very end; their length increases progressively along the whole length of body, attaining a maximum at the last segment, which is longer than broad. The genital pores are on the lateral margin and are irregularly alternate.

Host. *Dasyatis akaei* (Müller and Henle), spiral valve.

6. **Calliobothrium eschrechtii** (van Beneden, 1849).

Syn. *Acanthobothrium eschrechtii* van Beneden, 1849.
Onchobothrium (Call.) *elegans* Diesing, 1854.

The specimens were obtained from the spiral valve of *Cynias manazo* (Bleeker) in Tokyo, 24. v. 1907; in association with other cestodes (*Acanthobothrium, Calliobothrium, Rhynchobothrium, Phyllobothrium* and *Anthobothrium*).

External characters. Length 4—11 mm.; maximum breadth of the posterior segments, 0·25 mm. Head provided with four opposite bothridia measuring 0·44 × 0·15 mm. Each bothridium armed with two pairs of simple, claw-like, well developed, hollow hooks of equal size and similar shape; the outer hook of each side is slender and slightly curved while the inner hook is stout and strongly curved.

The hook is 0·12 mm. long measured along the curved surface and 0·03 in diameter at the base. Each bothridium is divided into three loculi by two transverse costae, the anterior loculus is largest, the posterior two sub-equal. Anterior to the hooks each bothridium is surmounted by a triangular pad with a single accessory sucker upon it. Posteriorly the bothridium is separated from the neck and slightly turned outward.

Neck slender, 1·2 × 0·1 mm. anteriorly immediately behind the head. Strobila generally delicate. The neck is followed by quadrate segments, 0·1 × 0·16 mm., which increase in size posteriorly, the length increasing more rapidly than the breadth, so that the last segments are much elongated, the terminal segment measuring 1·0—1·4 × 0·25 mm. In the posterior segments the genital openings are indicated by slight elevations which are readily seen at the posterior half of the lateral margin of each segment; they are irregularly alternate.

Linton recently obtained this species from the spiral valve of *Mustelus canis* at Woods Holl; my specimens are identical with those Linton described, differing only in the dimensions of various parts:

	Head length	Bothrid. length	Bothrid. breadth	Hook length	Neck length	Neck breadth	Last segment length	Last segment
Linton	0·9	0·6—0·64	0·34	0·20—0·24	—	0·2—0·24	1·0	0·32—0·6
Mihi	0·5	0·44	0·15	0·12	1·2	0·1	1·0—1·4	0·24

The above table shows that Linton's specimens are as a rule larger (measurements in mm.).

7. **Calliobothrium verticillatum** (Rud., 1819) van Beneden, 1850.

Syn. *Bothriocephalus verticillatus* Rudolphi, 1819.
Onchobothrium verticillatum Rud. of Diesing, 1850.
Acanthobothrium verticillatum (Rud., 1819) van Beneden, 1849.
Tetrabothrium verticillatum (Rud.) Wagener, 1854.

This species is very common in the spiral valve of *Cynias manazo* (Bleeker) in Japan. My specimens were obtained on several occasions at Tokyo, and other parts of Japan, and were generally found associated with other cestodes. In May, 1913, numerous specimens were collected at Nakatsu. They were much larger than any specimens previously collected at either Tokyo or Hiroshima, all measuring about 140 mm. in length. The external characters and internal structures of my

specimens agree well with those of *C. verticillatum* (Rud.); the only differences lie in the dimensions of the various parts of body and the number of segments; my largest specimens consist of 580 segments. Such a number of segments has not so far been recorded.

The anterior part of body is characteristically feeble and filiform, and so delicate that careless collectors usually leave the head attached to the host. The segments grow progressively in length posteriorly, the last segment being longest; the flaps on the posterior borders show slight individual differences; my specimens mostly differ from those previously described in respect to the flaps.

The first segment bears four triangular flaps on each corner of the postero-lateral margin. In the 65th segment, the middle of the posterior margin begins to protrude and soon after it becomes the third flap. This condition (laciniated border) of the segment continues down about 90 segments until about the 150th segment, in which the third flap begins to notch at its tip so as to form a bifid flap; the notch then becomes deeper and deeper, and ultimately the third flap divides into two; when this stage is reached, the posterior margin of each segment bears eight flaps, four on each, situated ventrally and dorsally. Of the four lateral flaps on each flat side, two are a little larger and sharper than the median pair. This arrangement of the flaps prevails in most parts of the strobila. At about the 425th segment, the median flaps become indistinct and are represented only by a slight flexure of the posterior margin. The notch between the two primary flaps is shallow and wide, but it becomes gradually deeper posteriorly as the median flaps disappear. In the last segment, the primary flaps become rounded and convex on its inner side. The dimensions of various parts of a worm, which measures 150 mm. in length and is composed of about 580 segments, are given (in mm.) in the following table:

	Head	Bothr.	Hook	1st segmt.	85th segmt.	120th segmt.	150th segmt.	225th segmt.	425th segmt.	last segmt.
Length	0·35—0·38	0·33	0·1	0·1	0·18	0·18—0·20	0·22	0·18	0·1	2·2
Breadth	0·38	0·12	—	0·11—0·16	0·20	0·4	0·5	0·8	0·7	1·1

The measurements taken from a smaller worm (45 mm. long) are as follows:

	Head	Hook	Bothrid.	Anterior segment	Middle segment	Last segment
Length	0·35	0·1	0·35	0·2	0·3	2·0
Breadth	0·25	—	0·14	0·06	0·2	0·75

8. **Calliobothrium convolutum** n. sp.

(Pl. XXIII, figs. 14—19 and Text-fig. 2.)

This species is very commonly found in the spiral valve of *Cynias manazo* (Bleeker) in Japan. The specimens which I examined were obtained from the shark, chiefly on three occasions, viz., 24. and 27. iv., and 9. v. 1907, in Tokyo. I have frequently found them associated with other species of cestodes, some of which I have already described.

External characters. Total length 55—110 mm. Head subquadrate and provided with four bothridia; its length and breadth vary naturally according to the state of contraction, on an average it measures ca. 1·0 × 0·7—0·8 mm.

In my largest specimens, the head measures 1·5 × 1·0—1·2 mm. The bothridium is elongated oval in shape, measuring 0·6—0·7 × 0·3—0·35 mm. Each bothridium turns outward but not forward, and its face is divided into three loculi by two transverse costae, the anterior costa lying in the middle or a little posterior to the middle of the bothridium; the second costa near the posterior end. Therefore, the anterior loculus is the largest and the posterior the smallest. The wall of the bothridium is somewhat thickened. Each bothridium bears one pair of thorn-like simple dark brown hooks (Pl. XXIII, fig. 15), the one being much larger than the other; the larger hook measures 0·3 × 0·1 mm. (basal width), and the smaller 0·17 × 0·06 mm. The paired hooks are closely apposed basally. Each hook is accompanied by a small process situated externally to its base and embedded in the tissue so as to be invisible from exterior.

In front of the hooks each bothridium is surmounted by a subtriangular pad, bearing a small accessory sucker anteriorly. The head is separated from the rest of body by a distinct neck, which may measure 2 × 0·5—0·7 mm., but its shape varies greatly in life.

In the strobila the breadth of the segment is always greater than its length; the breadth gradually increasing toward the middle of the body where it may reach a maximum along a succession of segments after which the breadth again diminishes slightly toward the posterior end. The length of the segments increases gradually and continuously from anterior to posterior end of the strobila. Actual measurements of length and breadth are slightly variable according to the different individuals or to the condition even in the same individual, but in the latter case there is no remarkable variability. Sometimes, though very rarely, we may find that the length is greater than the breadth in the

last few segments. In some specimens the anterior segments measure 0·05—0·085 × 0·8—1·3 mm. (the proportion being as 1 : 15 or 1 : 16). The widest middle segments measure 0·4 × 3—4 mm. (1 : 75—1 : 10), and the posterior segments, which are narrow and elongate, measure 0·85 × 1·7 mm. (1 : 2) or 1·1—1·5 × 2·0—2·2 mm. (1 : 15—1 : 2).

The anterior and posterior parts of the strobila can readily be recognized at a glance. Anteriorly the segments are much broader than long, their surface is thin and smooth, and the posterior border

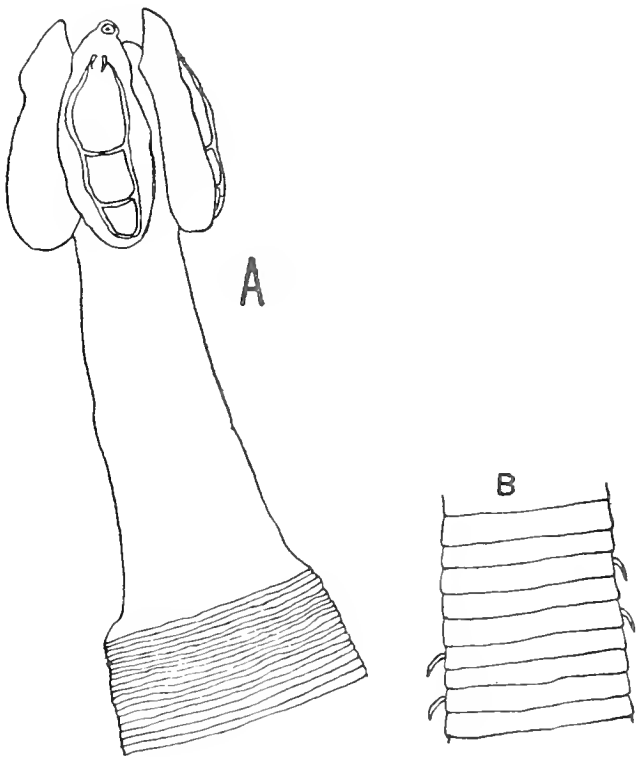


Fig. 2. A. Anterior end. B. Anterior segments. × 10.

of each segment more or less overlaps the following segment, giving a serrate appearance to the lateral margins. Posteriorly the segments become thickened by the accumulation of the ova in uterus, and narrowed at both the anterior and posterior borders, hence the serrated appearance is entirely lost. The most remarkable and peculiar feature of this species lies in the fact that some posterior segments fold ventrally upon themselves along the lateral margins. Posteriorly the folding becomes more and more pronounced and finally both margins meet each other along the ventro-median line making the

worm tubular (Pl. XXIII, fig. 14). Such peculiar terminal segments are somewhat thinner than those that precede them. The flattening of the posterior segments may be due to the discharging of ova from the uterus judging from the fact that the uterine openings are very conspicuous, forming macroscopically visible spots ranged along the ventral median line.

The cirrus openings are irregularly alternate, situated at the middle or a little anterior on the segments. The cirrus is frequently protruded from its pore; it is long, slender, and unarmed. The uterus openings are situated on the ventro-median line, a little anterior to the middle of segment.

INTERNAL STRUCTURE. The parenchymatous tissue is very loose and the cuticular layer is very thin. There is no sharp line of demarcation between the marginal layer and central core, that is, the transverse muscle fibres separating the two fields are very weakly developed. The longitudinal muscle fibres are also not conspicuous. I have found a few bundles of them here and there running through the marginal layer in horizontal and sagittal sections, but it is difficult to find them in cross sections. The fact that this species varies slightly in both length and breadth may be explained by the feebly developed musculature. In the anterior immature segments, the marginal layer is tolerably thick measuring about one-fourth the body thickness, but in the mature segments the central core bulges out the marginal layer by the growth of the reproductive organs, especially the uterus.

EXCRETORY CANALS. As a rule there are two main excretory canals on each side, dorsal and ventral, running throughout the whole length of worm, just inside the yolk glands; the canals are small but distinct.

FEMALE ORGANS. The vagina opens into the common genital cloaca on the antero-dorsal side of the cirrus opening. From the opening, the vagina proceeds inward straight in front of the cirrus pouch. Near the base of the pouch, the vagina rapidly widens, and bends on itself dorso-posteriorly along the basal end of the pouch; and passes dorsally to the excretory canal on the porose side to again run inward to the median part of the segment, where it coils on itself; ultimately it becomes the seminal receptacle, which lies on postero-ventral side of the segment. The proximal end of the seminal receptacle continues to the small duct, which runs dorsally over the ovary and turns again ventrally to unite with the oviduct coming from the ventral side. The united duct runs dorsally to open into the shell gland (Pl. XXIII, figs. 16 and 17). The main portion of the vagina, between its opening and the seminal

receptacle, possesses a nearly uniform diameter of 0·05 mm. The seminal receptacle measures 0·09 mm., and the proximal part of the vagina gradually narrows from the seminal receptacle to the point where it joins the oviduct. The entire length of the vagina is surrounded by a single layer of cells with distinct nuclei.

The oviduct (Pl. XXIII, figs. 16 and 17, *O*) begins with the so-called "egg-swallower" (Pl. XXIII, figs. 17 and 19, *E*) which is continuous with the middle portion of the ovary ventro-posteriorly. The "egg-swallower" is a remarkable organ, shaped like a sphere flattened antero-posteriorly; its lumen is spacious and its muscular wall is very well developed; the radial muscular fibres are the most highly developed. Just outside the muscular wall, the "egg-swallower" is surrounded by a thick layer of distinctly nucleated cells (Pl. XXIII, fig. 19). The oviduct, which follows, runs dorso-ventrally in a pronounced curve within the medio-posterior part of segment. The width of the oviduct is 0·035 mm. proximally, and it gradually broadens distally, its end measuring 0·05 mm.; it narrows again at its junction with the vagina. The lumen of the oviduct also widens gradually from the proximal to the distal end. The wall of the oviduct consists of a homogeneous membrane and two layers (inner thicker and outer thinner) of distinctly nucleated cells; the innermost membranous layer of the wall is provided with minute spinules projecting backward into the cavity. In the outer thinner layer the cell boundaries are not conspicuous.

The shell gland (Pl. XXIII, figs. 16 and 17, *SD*) is spherical or somewhat flattened in shape, measuring 0·2 to 0·14 mm.; it is situated medio-dorsally, posteriorly in each segment; it is composed of spindle-shaped cells with well-defined nuclei which are readily seen under a moderate magnification. At its centre the shell gland receives the yolk duct coming from the ventral side.

The yolk duct has a diameter of 0·007 mm. and bifurcates at the point where it passes across the middle of the ovary, the branches of the duct proceeding right and left to unite with the yolk glands which are situated just outside the excretory canal on each side of body (Pl. XXIII, fig. 16, *D*). The yolk cells are very small, measuring 0·0011—0·0013 mm. in diameter. By the smallness of the yolk cells we can easily distinguish them from the ovarian eggs although both of them look alike.

The ovary (Pl. XXII, figs. 16 and 17, *K*) is irregularly lobed, situated in the posterior part of body, spreading from the median plane to the lateral excretory canals. It lies mainly on the ventral side of the segment, but it extends out to the dorsal, thus occupying an extensive

arca at the posterior portion of segment. The lateral halves of the ovary are connected by a comparatively narrow isthmus in the median plane, where the ovary is continuous with the "egg-swallower" whose opening into the ovary is best seen in horizontal and sagittal sections. The ovum in the ovary measures 0·013 mm. in diameter and its nucleus (0·005 mm.) can easily be observed in haematoxylin-eosin preparations.

The uterus (Pl. XXIII, fig. 16, *U*) first makes its appearance transversely on the ventral side of the segment, and later its cavity enlarges more and more as it receives more ova; in gravid segments, it occupies all the available space, the other genital organs being atrophied. It is surrounded by a thin membrane, composed of cells with well-defined nuclei. It opens to the exterior a little anterior to the middle in the medio-ventral line of the segment (Pl. XXIII, fig. 14).

Male organs. The testes (Pl. XXIII, fig. 16, *H*) are numerous and scattered chiefly anteriorly and dorsally to the female organs; they are oval or spherical in shape, measuring 0·11 × 0·05 mm. The vas deferens is thin-walled and coiled on itself, more so near the base of the cirrus pouch; it is enlarged by being filled with spermatozoa in the mature segments. On entering the pouch it widens (0·04—0·105 mm.) to form a vesicle-like duct and it is coiled two or more times inside the base of the pouch; the coiled part of the duct is thin-walled, but its distal end is straight, narrow (0·025 mm.) and has a thick muscular wall. The wall of the vas deferens in the pouch is surrounded by a single layer of clearly nucleated cells, like those in the vaginal wall (Pl. XXIII, fig. 18, *L*). The protruded cirrus attains a length of 0·4—0·6 mm.

The cirrus pouch (Pl. XXIII, fig. 18, *B*) is elongate pyriform, it measures 0·5 × 0·15 mm. basally, and is 0·09 mm. wide distally. Its wall is thin and surrounded by a layer of cells with distinct nuclei. The space between the vas deferens and the wall of the pouch is filled with parenchymatous tissue containing scattered nuclei. The cirrus pouch with the vagina passes dorsally to the excretory canals on the porose side.

Affinities. There are some difficulties in deciding to which genus the worm belongs. The characters of the bothridium and the accessory sucker, the size, form and arrangement of the hooks offer important criteria for the systematic determination of *Phyllacanthinae*. Some differential characters of three genera are here tabulated (other genera of *Phyllacanthinae* may be omitted because of their being decidedly different).

Genera	Bothridium	Hook	Access. sucker
Calliobothrium	Each bothrid. provided with 3 loculi	4 single to each bothrid.	1 or 3 access. suckers in each bothrid.
Onchobothrium	Do.	2 single hooks whose base is continuous	No access. sucker
Acanthobothrium	Do.	2 bifurcated hooks (1 pair to each bothrid.)	1 or 3 access. suckers to each bothrid.

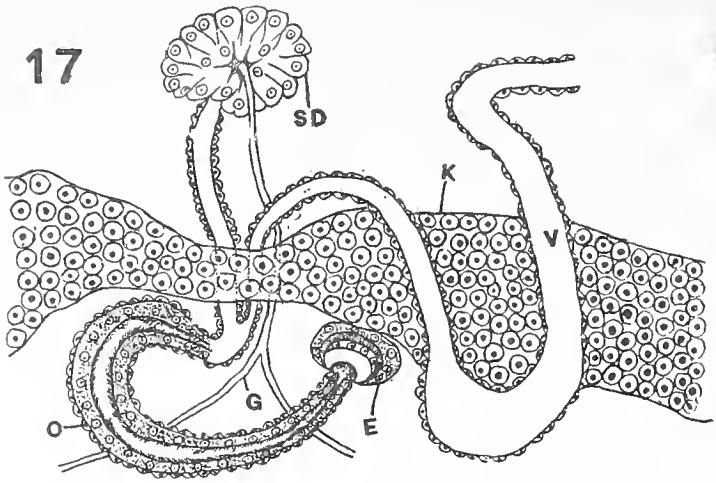
From the above table, it will be seen that our worm differs from two genera, *Calliobothrium* and *Acanthobothrium*, by the number and form of the hooks, but agrees with them in the feature of the bothridium and in the possession of one accessory sucker. The worm agrees with *Onchobothrium* in the character of bothridium and in the number and form of the hooks, but it differs from this genus by the presence of an accessory sucker and by the paired hooks being connected basally. Thus, so far as the characters of the head are concerned, it is difficult to refer it to a known genus. At first I thought of establishing a new genus for it, but now prefer to place it in the genus *Calliobothrium* because:

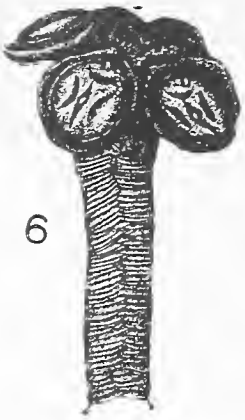
(1) The features of bothridium and the presence of one accessory sucker agree with the generic characters; (2) in this genus, we find that the hooks vary in number and shape (e.g., *C. aetiobatis* Shipley, from *Aetiobatis narinari* Euphras., is provided with a single pair of bifurcated hooks; *C. farmeri* Southwell, from *Trygon walga*, is provided with a single pair of hooks); (3) each of the two hooks has a small lateral process, which may represent an independent hook in *Calliobothrium*; (4) all the other generic characters agree.

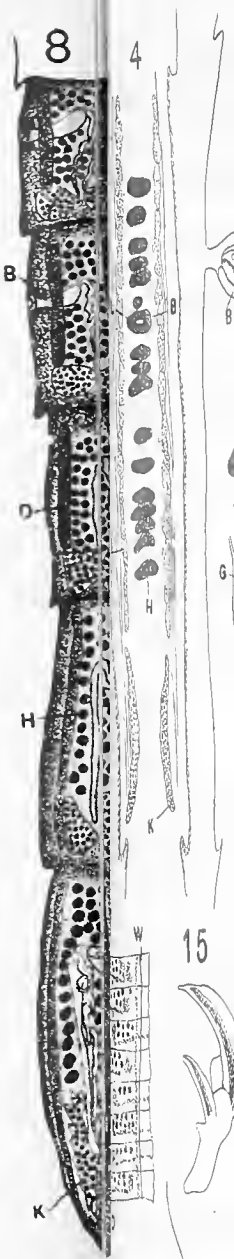
Besides the characters above enumerated the folding of the posterior segments differentiates our worm from any other species belonging to the genus.

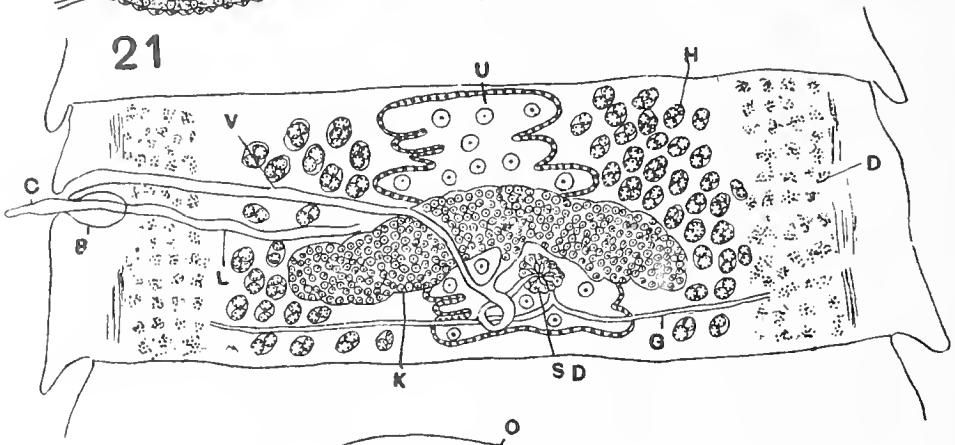
DIAGNOSIS. Length 55—80 mm. or 110 mm. Head subquadrate 1·0—1·5 × 0·7—0·8 or 1·2 mm. Bothridia four in number, subelliptical, each divided into three loculi by two transverse costae, the anterior loculus largest, the posterior smallest. A single pair of simple hooks on each bothridium, anteriorly on the anterior loculus; hooks dark brown in colour, thorn-like in shape, the one hook slightly larger; each hook accompanied by a small lateral process embedded under the surface and invisible externally. One accessory sucker situated on the anterior subtriangular pad of each bothridium. Head distinctly separated from strobila by the neck which is tolerably long.

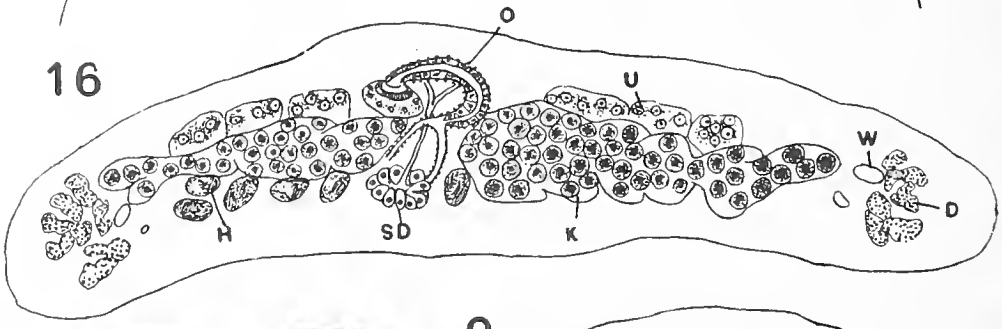
Segments always broader than long; their breadth gradually

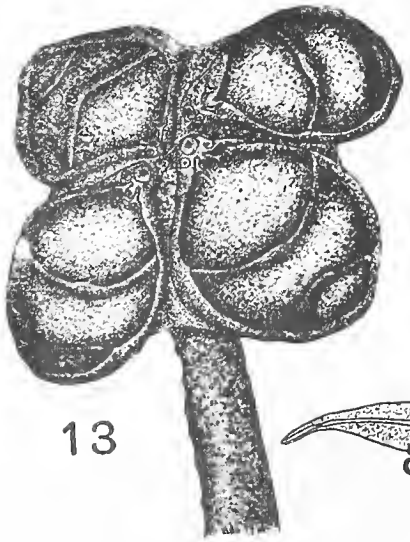


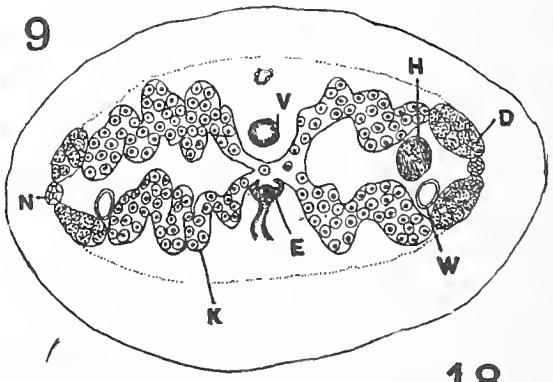


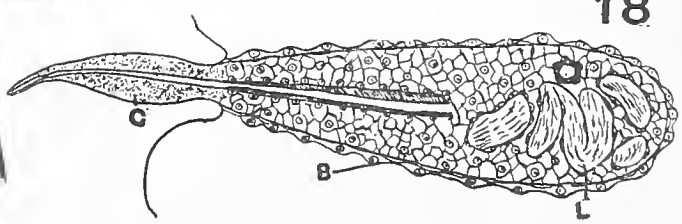


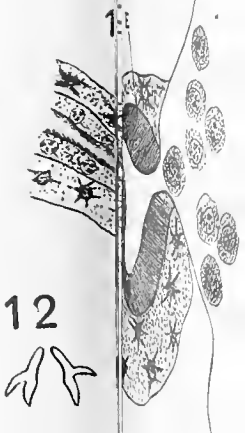












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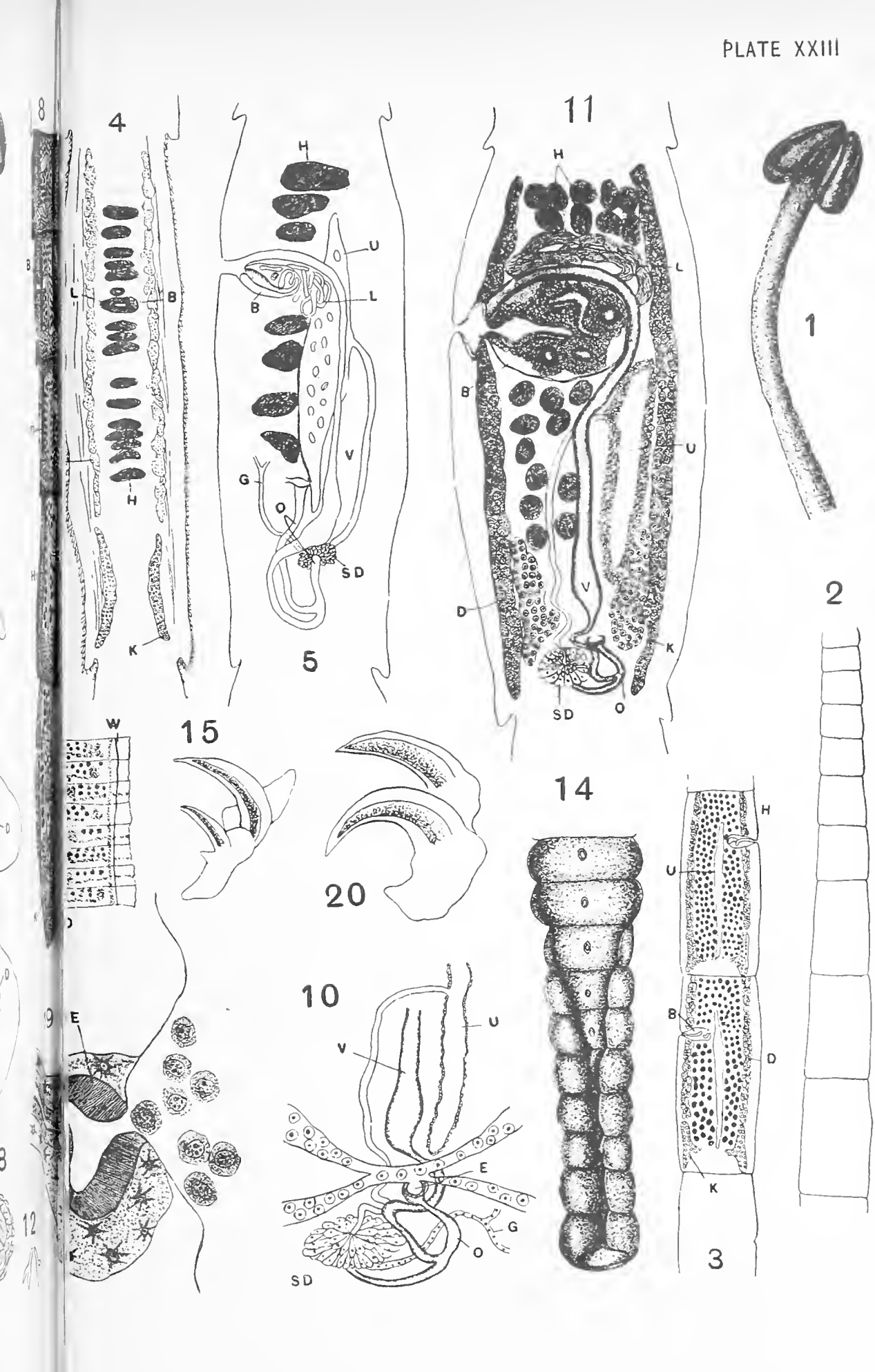
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increases toward the middle portion of body, reaching a maximum of 3—4 mm., and diminishing toward the posterior end; this length gradually increases toward the last segment, attaining a maximum of 1·0—1·5 mm. Anteriorly the strobila is thin and smooth, posteriorly it is swollen and folded to make a tube.

Genital openings lateral and irregularly alternate, situated slightly anterior to the middle of each segment. Uterine pores on the ventral median line.

Musculature weakly developed. Excretory canals two per side, running just inside the yolk glands.

Male organs. Testes numerous, situated antero-dorsally to female organs, oval or spherical, measuring 0·11—0·15 mm. Vas deferens much coiled on itself near the outside of the basal portion of cirrus pouch, entering the pouch it enlarges to the vesicle-like duct; its distal portion is straight and thick-walled. Cirrus pouch elongate pyriform, measuring 0·5 × 0·15 mm. basally and 0·09 mm. broad distally. Vas deferens and cirrus pouch surrounded by cell layer with distinct nuclei. Male duct with vagina crosses the longitudinal canals dorsally.

Female organs. Ovary irregularly lobed, extending transversely between the longitudinal canals on either side in posterior part of segment; lying mainly ventrally, but extending dorsally. Ovary united to oviduct by the so-called "egg-swallower" in the median plane. "Egg-swallower" subspherical, depressed antero-posteriorly, its wall with strongly developed radial muscle fibres surrounded by cell groups. Oviduct follows "egg-swallower," its course is strongly curved dorsally to union with proximal end of vagina. Oviduct provided internally with minute spinules, surrounded by two cell-layers with well-defined nuclei. Shell gland spherical or slightly flattened, measuring 0·2 × 0·14 mm., dorsal to the ovary and on the same level. Yolk duct arising at centre of gland, running ventrally and passing posteriorly to ovary, then crossing ovary and bifurcating, both ducts running outward to meet the yolk glands. Yolk glands situated just outside lateral excretory canals. Vagina runs from its opening inward straight to anterior edge of cirrus pouch; after crossing the longitudinal canals dorsally, it runs to median portion of segment, where it coils several times, and soon enlarges to form the seminal receptacle; the small duct runs dorsally and again bends ventrally to union with oviduct. Vagina surrounded throughout by cell-layer with well-defined nuclei.

Host. *Cynias manazo* (Bleeker), spiral valve.

9. **Calliobothrium nodosum** n.sp.

(Pl. XXIII, figs. 20, 21, and Text-fig. 3.)

The material was found with other cestodes in the spiral valve of *Cynias manazo* (Bleeker) 15. v. 1906.

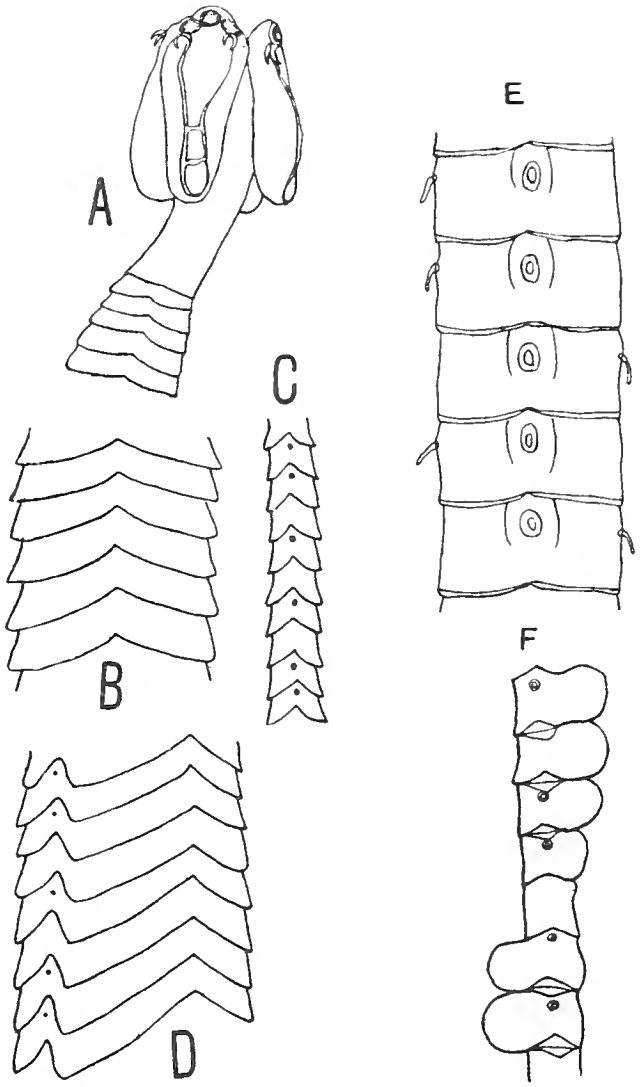

Fig. 3. A. Scolex with the anterior part of the strobila. B. Anterior segments (flat view). C. The same (side view). D. Middle segments. E. Posterior segments. F. Segments near the posterior end. × 10

EXTERNAL CHARACTERS. The largest specimens measure 160 mm. in length. Head subquadrate and provided with four bothridia, which are placed diagonally, neither horizontal nor saggital. Bothridia

large and elongate oval, each measuring 1·1 × 0·6 mm., divided into three unequal loculi by two transverse costae. The anterior loculus is the largest and at its anterior corner two pairs of hooks are present. Hooks thorn-like, strongly curved, measuring 0·4 × 0·5 mm. (basal width), the pair on the same side of the bothridium are connected by fibrous tissue. In front of hooks, bothridium carries a pad with three accessory suckers. Neck varies in length according to state of contraction, measuring 1·3—1·5 × 0·8 mm. (average width).

The strobila of the largest specimen is widest (4·5 mm.) near the posterior end, narrowest (0·8 mm.) at the neck, the last segment measuring 1·5—2·0 mm. in breadth. The strobila is nearly of uniform thickness (1·0 mm. or more) excepting at the posterior part of the body which is nodularly swollen by the distended uterus (fig. 3, *E* and *F*).

The segments increase gradually in length toward the posterior end, the anterior segment measures 0·2 mm., the middle 0·5 mm. and the posterior 1·0 mm. long. The segments also increase in breadth posteriorly to near the end of the body where they again narrow at the very end. The posterior segmental border is angular or laciniated by emarginations as shown in the accompanying figures; the laciniation differs slightly in different parts of body; the emarginations of the posterior border are generally four in number, two on the dorsal and ventral median lines and one on each side of segment. The posterior border overlaps the next following segment so as to give a serrated appearance to the worm. Serration, emargination or laciniation are conspicuous in the anterior half or more of the worm and are obliterated in the rest of the strobila.

Genital pores irregularly alternate, situated in front of the middle of the lateral margin. Uterine openings placed on median line of the flat surface.

Excretory system. Dorsal canals very small, the ventral larger, measuring 0·046 × 0·034 mm. Wall of ventral canal well developed, 0·005 mm. in thickness. Transverse commissures of ventral canals absent.

Musculature. Large longitudinal muscle bundles (ca. 100) are somewhat regularly arranged all around the median field. The anterior portion of body is furnished with fewer muscle bundles, but they are well developed (each bundle measuring 0·041 mm. in long and 0·02—0·03 mm. in short diameter).

Male organs. Cirrus pouch (Pl. XXIII, fig. 21, *B*) oval, 0·28 × 0·17 mm., surrounded by a thin muscular wall; its cavity is occupied

by parenchymatous tissue in which the vas deferens and cirrus are embedded. The vas deferens in the cirrus pouch passes straight toward the opening of pouch, and becomes a cirrus, which is protrusive. In the anterior segments, the vas deferens coils somewhat in the basal part of the pouch. Leaving the cirrus pouch, the vas deferens runs inward to the median line of the segment taking a slightly winding course, nearly parallel to the vagina (Pl. XXIII, fig. 21, *L*). The further connection to the testes could not be traced. The vas deferens measures 0·02 mm. in diameter within the pouch and 0·022 mm. in diameter outside it.

Testes (Pl. XXIII, fig. 21, *H*) numerous, scattered chiefly dorso-laterally and a few posterior to female organs; oval in shape, measuring 0·115 × 0·08 mm.

FEMALE ORGANS. Vagina (Pl. XXIII, fig. 27, *V*) opens into common genital cloaca immediately antero-dorsal to the opening; from its orifice it runs inward, then bends backward to unite with the oviduct, coils once, and runs forward to open into the shell gland. The transverse portion of the vagina is of nearly uniform diameter (0·03 mm.), the proximal portion narrows (0·017 mm.); it is lined by a single cell-layer and surrounded by a thick cell-layer.

Ovary (Pl. XXIII, fig. 21, *K*) occupies nearly all the median posterior ventral half of the segment. The oviduct arises midway along the ovary and runs back to unite with the vagina; at its origin is a spherical "egg-swallower" with a muscular wall (0·01 mm. in thickness); oviduct wall of similar structure to vagina. *Egg* (ovarian): spherical or oval, 0·02 × 0·013 mm. with nucleus measuring 0·0115 mm. Shell gland (Pl. XXIII, fig. 21, *SD*) situated posterior to the ovary; oval, measuring 0·1 × 0·065 mm.

Yolk glands (Pl. XXIII, fig. 21, *D*), numerous, longitudinally arranged outside the excretory canals; their main ducts (*G*) run inward posteriorly in the segment, meet in the median line and open into the shell gland by a short duct. Uterus (*U*), lies in the median portion of segment; in anterior segments it is only represented by cell groups within which a lumen appears that subsequently enlarges, ultimately, in the posterior part of the strobila, occupying almost all the median portion of the segment. In mature segments the uterus swells out rendering the segments globose (Text-fig. 3, *F*). The uterine ovum, with shell, is nearly spherical and measures 0·034 mm.

AFFINITIES. The external features of the scolex, such as the number of the hooks, and the bothridium form, are as in the genus *Calliobothrium*,

but the internal structures, especially the genital organs, differ from those of the genus and resemble more those of *Onchobothrium*. The laciniation of the posterior segmental border and the swelling of the posterior gravid segments are characters sufficient to distinguish the worm from any known species of *Calliobothrium*.

Diagnosis. Length 100—160 mm. or a little more. Head subquadrate, with four bothridia situated diagonally. Bothridium large and elongate oval, measuring 1·1 × 0·6 mm.; its face divided into three loculi by two transverse costae, the anterior loculus is the largest, the other two are of subequal size. Hooks: two pairs of simple hooks at the anterior corner of anterior loculus; they are strongly developed and thorn-like in shape. There are three accessory suckers. Neck variable in length and breadth. Strobila nearly uniform in thickness (1·0 mm. or more) throughout most of the body, but the posterior gravid segments are swollen so as to give a nodular appearance. Segments: their length gradually increases caudally; maximum length in the last segment; maximum breadth near the posterior end; the breadth decreases posteriorly; posterior segmental border laciniated by four emarginations, two lying on the median line of the flat surface and two on the lateral margins. Cirrus openings, on lateral margin, irregularly alternate. Uterine pores situate along median line of flat surface.

Cirrus pouch small, oval, measuring ca. 0·28 × 0·17 mm. Vas deferens nearly straight from cirrus pouch to median plane. Testes oval, 0·15 × 0·08 mm., scattered chiefly dorso-laterally to female organs, but a few posterior thereto. Vagina runs nearly parallel and dorsally to vas deferens, proximally it unites with the oviduct and runs to the shell gland. Ovary situate ventrally, occupying nearly all the median posterior half of segment. Yolk glands occur in numerous groups arranged longitudinally outside the lateral canals on both sides. Uterus in the median portion of segment; it swells considerably in the posterior or gravid segments.

Host. *Cynias manazo* (Bleeker), spiral valve.

10. **Rhynchobothrium laciniatum** n.sp.

(Text-figure 4.)

A few specimens of this species were obtained from the spiral valve of *Cynias manazo* (Bleeker) on May 1913, at Nakatsu. They were found associated with other species of cestodes.

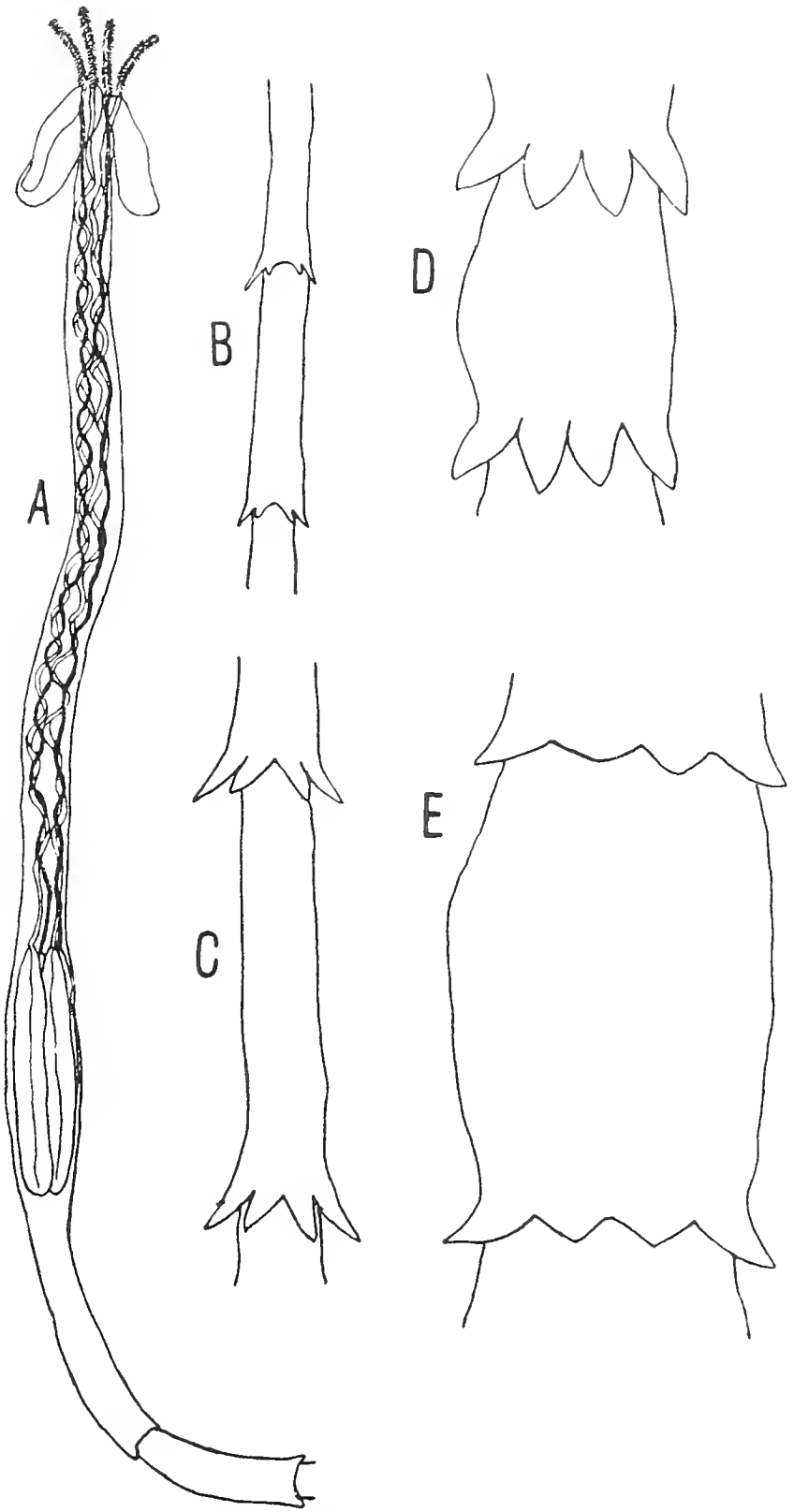

Fig. 4. A. Scolex and anterior segments. × 25. B. 5th segment. × 25. C. 20th segment. × 25. D. 45th segment. × 10. E. Last segment. × 10.

External characters. Total length 100 mm.; number of segments ca. 70. Head provided with two oval bothridia, measuring 0·33 × 0·27 mm.; connected along their anterior half to the head axis, directed antero-laterally, very movable in life; with two openings anteriorly, from which long proboscides protrude. Neck fairly long (3 mm.); breadth 0·13 mm. just behind the head, 0·1 mm. (minimum) anterior to proboscis sac; proboscis sac region slightly swollen, spindle shaped (0·5 × 0·2 mm.), continuous behind with the first segment (0·1 mm. broad); anterior 20 mm. of strobila, comprising ca. 20 segments, is very slender and delicate in texture; the segments then gradually lengthen and widen proceeding backward, the last segment being largest. The dimensions of various parts of body are given below (in mm.):

	Bothrid.	Neck	Probosc. sac	1st segmt.	5th segmt.	20th segmt.	40th segmt.	45th segmt.	Last segmt.
Length	0·33	3	0·5	0·35	0·7	1·6	1·4	1·5	2·9
Breadth	0·27	0·1—0·13	0·2	0·1	0·1	0·2	0·6	1·2	1·9

Segments, always much longer than broad; anteriorly along the strobila the segments are broadest at their posterior borders whilst in the posterior region of the strobila they are broadest in the middle; each segment bears pointed triangular flaps postero-laterally projecting from its posterior border.

There are usually six flaps per segment but a few anterior segments bear two or six, where they bear two the flaps are small, one projecting on each side of the body; further back, four small secondary flaps appear dorsally and ventrally to the primary flaps; the flaps gradually increase in size. At the 20th segment the secondary flaps are nearly as large as the primary ones, then all six flaps increase equally in size caudally, reaching their maximum dimensions (0·55 mm.), at the 40th segment. In a few segments, following upon the 40th, the flaps are of uniform size, after which they shorten gradually and become smaller and obtuse. Genital openings lateral, irregularly alternate.

Genital organs. Testes distorted oval or elliptical; scattered laterally in medullary field; five to seven or more testes visible in cross section of the segment; most testes usually in the aporose half. Cirrus pouch large, elongated pyriform in shape, extending beyond middle of segment; occupying nearly all the porose half of the medullary field, leaving the narrow space at its ventral side where the vagina runs transversely parallel to the pouch; with thick muscular wall. Vas deferens entering the pouch basally, is narrow and strongly coiled in the

base of the pouch, then runs straight (broadening, especially distally) outward to the exterior; deep genital cloaca absent; muscular wall of duct uniformly thick.

Ovary voluminous, situated in middle region of segment, extending dorso-ventrally to occupy the whole thickness of medullary field; divided into two lobes, right and left. Yolk glands numerous, arranged in circular row between the cortical and medullary fields.

DIAGNOSIS. Length 100 mm. or a little more. Head provided with two bothridia, measuring 0·33 × 0·27 mm. Neck tolerably long. Proboscis sac spindle shaped, 0·5 × 0·2 mm. Strobila slender and delicate anteriorly, thick in the middle and behind. Segments always longer than broad; their posterior border with six triangular characteristic flaps, which gradually enlarge toward the middle of body, and thence decrease in size posteriorly. Genital openings lateral, irregularly alternate.

Host. *Cynias manazo* (Bleeker), spiral valve.

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EXPLANATION OF PLATE XXIII.

B.	Cirrus pouch	*H.*	Testis.	*SD.*	Shell gland.
C.	Cirrus.	*K.*	Ovary.	*U.*	Uterus.
D.	Yolk gland.	*L.*	Vas deferens.	*V.*	Vagina.
E.	Egg-swallower.	*N.*	Nerve.	*W.*	Excretory canal.
G.	Yolk duct.	*O.*	Oviduct.		

Figures 1—5.

Crossobothrium angustum Linton.

Fig. 1. Scolex. ×30.
Fig. 2. Middle segments. ×30.
Fig. 3. Posterior segments. ×30.
Fig. 4. Sagittal section through the posterior segment.
Fig. 5. Diagram showing the interrelation of the genital organs.

Figures 6—11.

Orygmatobothrium velamentum n.sp.

Fig. 6. Scolex and the anterior part of the body. ×20.
Fig. 7. Middle segments. ×20.
Fig. 8. Posterior segments. ×20.
Fig. 9. Cross section through the level of ovary.
Fig. 10. Diagram showing the interrelation of the female organs.
Fig. 11. Horizontal section (semi-diagrammatic).

Figures 12—13.

Acanthobothrium ijimai n.sp.

Fig. 12. Hooks. ×80.
Fig. 13. Scolex. ×24.

Figures 14—19.

Calliobothrium convolutum n.sp.

Fig. 14. Posterior segments. ×5.
Fig. 15. Hooks. ×65.
Fig. 16. Cross section through the level of the ovary.
Fig. 17. Interrelation of female organs.
Fig. 18. Cirrus pouch. ×120.
Fig. 19. Egg-swallower. ×300.

Figures 20—21.

Calliobothrium nodosum n.sp.

Fig. 20. Hooks. ×150.
Fig. 21. Matured segment.

NOTES ON NYCTERIBIIDAE, WITH DESCRIPTIONS OF TWO NEW GENERA.

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(With Plate XXIV.)

This paper is written primarily with the intention of publishing descriptions of two new genera of Nycteribiidae, together with some notes relating to certain already known forms of that family. But it also includes an account of some recent work (not my own), which for the first time gives us a detailed insight into the habits of these bizarre creatures. This latter side of the subject will be considered first.

A. BIOLOGICAL SECTION.

The summary of recent biological observations may be prefaced with some more general remarks.

Nycteribiidae are a family of highly modified and quite wingless Diptera, classed in the probably polyphyletic group "Diptera Pupipara." The components of this differ widely *inter se*, but all possess in common the characteristic of retaining the young within the body of the parent till larval life is practically completed, and of then giving birth to the matured larva, which almost immediately commences its transition into the pupal stage. Nycteribiidae, as well as another family of Pupipara, the Streblidae, are external parasites found exclusively on bats.

Geographical Distribution. Only a brief allusion will be made to this subject. Speiser tabulated the distribution of all known forms (1901, pp. 62–3[1]), and discussed the question further in two interesting later papers (1907, 1908[1]). His statements have necessarily been somewhat modified by subsequent findings, and our knowledge needs sum-

[1] References thus given are to the list at the end of the paper.

marising afresh before detailed conclusions are drawn. But some broad facts seem fairly established. As remarked by Speiser (1908, p. 423) the geographical headquarters of Nycteribiidae appear to be in the Old World, the lands bordering the Indian Ocean being particularly rich in species. By comparison, the family seems but poorly represented in America. The Streblidae, on the other hand, are much better represented in the New World, while possessing also a second centre in certain of the lands adjacent to the Indian Ocean.

Many Nycteribiidae have a very wide geographical range, which, as previously remarked (Scott, 1913, p. 93), is not surprising in view of their hosts' powers of distribution, and of the insects' power of attaching themselves to several different species of hosts (see below). The paper just referred to shows that certain species known from many parts of Europe and from N. Africa have been found also in Formosa, and may occur across all the intervening part of the Palaearctic Region; while other forms are widely distributed in the Oriental Region. *Eucampsipodia hyrtli* is known from Egypt, West and South Africa, Comoro Islands, Burma, Ceylon and Sumatra. *Tripselia fryeri* (discussed in this paper) was described from Assumption Island, to the North of Madagascar, and from Labuan; it has now been found in the Belgian Congo.

Relation of parasite to host. (i) A single species of Nycteribiid may infest several species of bats. (ii) Conversely, a single species of bat—even a single individual—may harbour several species of Nycteribiidae.

(i) Speiser's enumeration of species (1901, pp. 49–56), and subsequent work, show that it is quite frequent for a single species of Nycteribiid to have several species of hosts. In such cases the hosts may be several bats of the same genus, or may represent two or more genera, or may even belong to more than one family, though the last condition is probably rare. There do appear, however, to be some broad restrictions, *e.g.* the same species of Nycteribiid is not usually found on both fruit-eating and insect-eating bats. Thus, the genus *Cyclopodia* (*s. str.*) seems to be confined to frugivorous forms (Pteropidae; cf. Speiser, 1907, p. 23), and I am not aware that the large Eastern species, such as *C. sykesi* and *C. albertisi*, have been found on any host but the big "flying-foxes" (*Pteropus* spp.). Other forms of *Cyclopodia*, which, as shown below, should be placed on account of structural characters in distinct subgenera, break this rule. Thus *Cyclopodia roylei* has been recorded from two genera and several species

of the small insect-eating Vespertilionidae, and also from the Nycterid bat *Megaderma lyra*, which eats small Vertebrates. This last is one of the cases in which the hosts belong to more than one family. It is shown in a table which I drew up for some Ceylonese Nycteribiidae (1914*a*, p. 212). The same table includes another such case, that of the wide-spread *Eucampsipodia hyrtli*, which has been recorded from several species of the fruit-eating genus *Rousettus* (evidently its normal hosts), and also in a solitary instance from the Vespertilionid *Tylonycteris pachypus*. *Penicillidia dufouri* is known from both Rhinolophidae and Vespertilionidae (Speiser, 1901, p. 50), both of which families, however, belong to the insectivorous category. The insects' powers of selection of hosts seem fairly elastic.

(ii) Conversely, as stated above, one species of bat may harbour several kinds of Nycteribiidae. At least 9 species have been recorded from *Miniopterus schreibersi* in various parts of its wide range. Attention has previously been called to the fact that examples of several species and genera of Nycteribiids may be found *on the same individual bat* (Scott, *Nycteribiidae from Formosa*, 1908, p. 359, and 1913, p. 93). In this connection the habits of the bats must be taken into consideration. So far as known, the insects leave the bats only rarely and for short intervals (see below), therefore their chances of infesting several species of bats must depend on the extent to which these come in contact with one another. The big flying-foxes of the genus *Pteropus*, which eat fruit, rest, as far as I know, not in caves or buildings, but hanging from branches of trees, and consort in great numbers in particular places (called "camps" in Seychelles). Such bats are not likely to come into contact with bats of other families and genera: as far as I am aware, no Nycteribiidae have been found on them except certain large species of *Cyclopodia*, which, conversely, have not been taken from bats of any other genus. On the other hand the vast majority of the Formosan Nycteribiids referred to above were obtained from large numbers of *Miniopterus schreibersi* congregated in an old temple. Isolated individuals of two other bats, a *Myotis* and a *Pipistrellus*, hung among the *Miniopterus*; the parasites could easily wander from one species to another, and some specimens were collected from *Myotis* and *Pipistrellus*, though the collector (Hans Sauter) stated that when these latter were captured alone, they were usually quite free from Nycteribiids. Perhaps some kinds of bats are constitutionally less liable than others to become infested, even though they may be equally accessible to the parasites. Somewhat in contrast to the Formosan case is that of 5 species of Nycteribiidae collected

from 3 species of bats in a large cave at Hammam Meskoutine in Eastern Algeria (P. A. Buxton, *Ent. Record*, Vol. XXVI, 1914, p. 68). One species of bat, *Myotis oxygnathus*, harboured 3 species of Nycteribiids; the other two, *Rhinolophus euryale* and *Miniopterus schreibersi*, were infested by only one species apiece. Also, none of the 5 species of parasites was taken from more than one species of bat. But the *Rhinolophus* and *Miniopterus* were found almost solitarily, in parts of the cave removed from one another and from the *Myotis*, great numbers of which were congregated in one place; and it is precisely this last species which harboured 3 kinds of Nycteribiids. If this was the normal disposition of the bats when at rest, it helps to explain the distribution of the insects, on the assumption that the latter do not wander any distance from their hosts.

Recent biological observations. Apart from the broad facts of their larviparism and parasitism, the biology of Nycteribiidae has been hitherto very little known. Recently, however, a very interesting paper by Rodhain and Bequaert (1916) has been published, describing in detail the behaviour of one species, *Cyclopodia greeffi* Karsch (= *rubiginosa* Bigot[1]), in the Belgian Congo. These writers commence by summarising the earlier contributions of Westwood, Osten-Sacken, etc., but they do not appear to have noticed some valuable particulars *à propos* of certain Oriental forms contained in a paper by Muir (1912). It is intended here to give a *résumé* of Rodhain's and Bequaert's work, referring also to that of Muir for purposes of comparison.

Rodhain and Bequaert procured at Leopoldville a number of living fruit-eating bats, *Cynonycteris straminea* E. Geoffr., which almost always harboured abundant specimens of *Cyclopodia greeffi*, to which species alone do their statements refer. The bats proved easy to keep alive in cages of wire-gauze. During almost the whole day they hung motionless, head downwards, from wooden perches, but at night they climbed actively about. They were fed almost entirely on bananas. In one case the roof of the cage was glazed, so that the movements of the parasites on the bodies of their hosts could be watched. [Muir was less fortunate, since the forms observed by him lived on an insect-eating bat, *Miniopterus schreibersi*: many specimens of this were placed

[1] The insects were determined as *C. greeffi* from Karsch's description, published in 1884. Specimens, submitted to me later, proved to be identical with the type of *C. rubiginosa* Bigot (1891), with which I compared them. It is extremely probable that the two names are synonyms; *C. greeffi* was originally discovered on the same species of bat as Rodhain's and Bequaert's material.

in cages, but they refused to eat the insects given to them and died within 48 hours (1912, p. 352).]

The *Cyclopodiae* often remain motionless for hours together, buried in the host's fur, the head and thorax quite hidden and only the hind end of the abdomen visible. They do not seem to cause the bats very great discomfort, though after a bite from one of the insects a bat will often scratch itself with the hooks of its wings or the claws of its feet. The parasites do not pry into their hosts' heads, and when pursued they escape with surprising agility and hide in the long hair of the neck or under the wings. [The agility of living Nycteribiids has been noticed before, *e.g.* as mentioned in the case of *Tripselia fryeri* (Scott), 1914*b*, p. 163: and Mr F. M. Howlett tells me that he has remarked the extreme agility of *Cyclopodia sykesi*, in India.] They do not normally leave their hosts without very good reason: females do so for short periods in order to give birth to larvae; males were never seen to leave the host-bats, though they may pass from one bat to another in search of females [especially when the bats are almost or quite in contact.]

As a result of more than 50 dissections, *fresh blood was always found in the dilated part of the middle intestine*, which would seem to indicate that they feed very frequently. Neither intestinal nor coelomic parasites of the *Cyclopodia* were ever found in the course of these dissections.

Insects removed from the bats survived but a very short time, and could not be induced to feed. In a glass tube they rarely lived more than 12 hours, and males under these conditions fought and killed one another very quickly. [Howlett found that specimens of *C. sykesi* survive only a very short time when removed from their hosts, whether they be placed in glass tubes or confined in gauze cages.] Nevertheless, specimens newly emerged from their puparia have greater powers of resistance, which is important for them, as in this stage they must find and invade their hosts. But if they cannot do this within 48 hours, they perish. They belong to that narrow category of ectoparasites which are closely adapted to their hosts and scarcely able to survive without the latter.

It is not rare to observe the coitus of the *Cyclopodia* on the bats: the male climbs on to the female and curves the extremity of the abdomen under the anal segment of the female, holding on to the latter by means of his powerful claspers. The coitus lasts at least 10 minutes.

Gravid females are distinguishable by the distension of the abdomen, which in a non-gravid condition is bluish-black, but when distended by

the larva becomes whitish. Then the insect leaves its host and runs rapidly about on the wooden perches to which the bats cling, and on the walls of the cage. From time to time it stops and raises its abdomen, which it brushes with its hind legs. Having at length discovered a suitable place in which to deposit its larva, the female stops, and, keeping the thorax still, moves the abdomen several times from left to right: at the same time it rapidly expels the larva, which by constriction and stretching passes through the narrow genital orifice and then almost instantaneously resumes its normal shape.

The larva does not move about after birth, but immediately adheres to the substratum on which it is laid. The female also immediately moves backwards, places itself over its progeny, and by alternately raising and lowering itself presses the ventral surface of its thorax closely against the larva. By this means the latter is, as it were, gummed to the substratum, from which it can only with difficulty be detached undamaged. The female repeats this pressing movement three or four times, then stays a moment uplifted on its legs, after which it runs quickly back to its host.

Puparia were never found on the bodies or in the excrement of the hosts: the numerous examples were all laid close to the bats, in most cases on the lower surface of the wooden perches from which the latter hung, sometimes on the wire-gauze or glass walls of the cages. Smooth and dry surfaces seem to be preferred for the deposition of the larvae. In a natural state the bats sleep suspended from branches, particularly those of *Dracaena*, on the smooth branches and trunks (perhaps also leaves) of which the larvae are probably laid.

[At this point it is important to compare the observations of Muir (1912, pp. 357–8), which show that at any rate some forms of Nycteribiidae fasten their larvae to their hosts. His observations are concerned with the kind described below as *Eremoctenia progressa*, referred to by him as *Penicillidia progressa*, and may be cited as follows: "The full-grown larva, when passing out of the uterus, becomes greatly flattened, especially on the ventral surface, and is held by its anterior end for a short time between the external flaps of the vagina, its ventral surface being pressed against the skin of its host; generally near the junction of the wing-membrane with the body or limb. The chitinous exudation...first appears along the edges of the flattened ventral surface and fastens it to its host..."] Kolenati attributed to a Nycteribiid certain puparia which he found attached to the hairs of a *Vespertilio*, but proof is required that these were not puparia of a Streblid. As

stated by Rodhain and Bequaert, the puparia of *Lipoptena* and *Melophagus* adhere to the hairs of their hosts.

As far as can be judged from several experiments, the first larvae of the *Cyclopodia* are born from 8 to 11 days after the emergence of the ♀ from the puparium, and succeeding births are separated only by intervals of 2–6 days. The conditions under which the bats are living at any particular time seem to affect the fecundity of the parasites.

Immediately after birth the larva assumes a shape identical with that of the puparium. It is a soft, transparent, milky-white body, half ellipsoidal, with elliptic contour, with dorsal surface convex and ventral surface flat, the two surfaces separated by an angular margin. Although stuck to the substratum, it undergoes active internal movements in relation with the process of nymphosis. It seems to be born at a less advanced stage than the larvae of *Hippobosca* and *Melophagus*, which at birth are already much more like puparia. There are two pairs of spiracles only, both postero-dorsal in situation, one pair being about one-third the length of the body from the hind end, the other pair closer to the hind end.

In transforming to a puparium, the hardening and darkening of the convex dorsal surface is completed in 20 to 30 minutes after birth. On the flattened ventral surface the process seems to be slower, and internal movements can be seen through this surface for more than 48 hours after birth. The puparium is shown in profile in Rodhain's and Bequaert's paper, Fig. 4, p. 258, with convex dorsal and flattened ventral surface and narrow explanate margin. The curved suture in the antero-dorsal part, along which the operculum is detached at the emergence of the imago, is more distinct than the divisions of the segments.

[Here again Muir's words (*l.c.*) regarding *Eremoctenia progressa* may be cited for comparison: "the chitinous exudation that covers the soft larval skin, to form the puparium, first appears along the edges of the flattened ventral surface and fastens it to its host, then covers the dorsal surface, but does not appear on the ventral side [apart from the edges], that side remaining a soft membrane through which, if carefully detached from the host, the pupa can be seen developing. The larval spiracles remain distinct and stand up above the surface. No anterior pupal spiracles or 'horns' appear, but the pupal thoracic tracheae are attached to two spots on the inner surface of the operculum, and can be faintly discerned externally. The operculum is large, the posterior edge curving across the dorsal surface near the middle, slightly in front

of the anterior spiracles, and continuing along the sides to the front; no line of dehiscence runs towards the ventral surface. The position of the head of the pupa would prevent the use of a ptilinum, as the legs are folded over the head and thorax, the femorotibial joints meeting in the middle line (see Muir's figure 10, plate II). A movement of the legs would force off the operculum."]

The pupal stage of *Cyclopodia greeffi* was found to last from 12 to 16 days. At emergence the imago is pale and feebly chitinised, but otherwise this phase is outwardly precisely similar to the fully matured form. The ♂ and ♀ internal genital organs of the adult are described and figured. The two ovaries were always found in an unequal state of development, indicating that their functions do not correspond chronologically, though whether there is a regular alternation in the production of ova is not known.

B. SYSTEMATIC SECTION.

Genus **EREMOCTENIA**, gen. nov. (Pl. XXIV, figs. 1–5).

Diagnostic Characters. *Thoracic ctenidium* entirely absent in both sexes. *Abdominal ctenidium* also entirely absent in both sexes, its place taken by a few ordinary bristles. *Eyes* quite absent. *Tibiae* not ringed, not broad and flattened. *Metatarsi* long, approximately $\frac{2}{3}$ the length of the tibiae.

Description. The form on which this genus is founded has at first sight somewhat the aspect of a *Penicillidia*, from which genus it is however clearly separated by the absence of ctenidia and eyes. The *head-capsule* is of characteristic shape, swollen and bulbous behind, narrowed in front. I am convinced of the absence of *eyes* in both sexes after a careful examination with the compound microscope: when these organs consist of single facets and have no dark pigment beneath them they are very easy to overlook, but in the present case diligent search has quite failed to reveal them. *Thorax:* Fig. 5 is specially drawn to demonstrate the complete absence of *ctenidia*, the front and middle legs being held aside to show the space where the ctenidia normally lie: the only visible structures which could possibly represent them consist of a small series of fine and minute bristles on the lateral margin immediately in front of the base of the middle coxa, but these hardly seem to be in the normal position for a ctenidium. Further particulars as to the thorax are included in the specific description below. *Halteres*

flattened and scale-like. *Anterior coxae* not elongated. *Middle coxae* each with a small blackish area at the outer distal angle (see Fig. 5). *Femora* of average form, only moderately robust. *Tibiae* rather slender, not ringed, with about 6 obliquely transverse series of bristles on the distal half of the lower surface, in the proximal series fine and short, in each successive series towards the apex becoming longer and more robust, in the apical series long, stout, and curved (there is *not* a marked predominance of three or four bushy series of long, stout bristles, as in some *Penicillidiae*). *Metatarsi* of all the legs, as stated above, long and slender. *Abdomen* described below, under the species.

Larva and pupa, see below, in the specific description.

Type of the genus: *Eremoctenia progressa* (Muir).

Affinities. All other described genera of Nycteribiidae have both thoracic and abdominal ctenidia, with the exception of *Archinycteribia* Speiser (1901), which has no abdominal ctenidium. *Archinycteribia* has however no other special points of resemblance to *Eremoctenia*, for it possesses thoracic ctenidia and single-faceted eyes, and has all its metatarsi very short. Its single species, *A. actena* Speiser, also differs widely in specific characters from *Eremoctenia progressa*. In having no eyes and in the form of the legs the genus resembles the subgenus *Acrocholidia* of *Nycteribia*, but I doubt if there is really any close affinity between them. On the whole I should be inclined to place *Eremoctenia* nearer to *Penicillidia*, in spite of the absence of eyes and ctenidia. In specific characters certain forms of *Penicillidia* are not altogether unlike *Eremoctenia progressa*: *e.g.* Kolenati's figures (1863, Pls. X, XI) of *Penicillidia conspicua* Speiser (= *westwoodi* Kol. nec Guér.-Mén.) show certain resemblances to *E. progressa* in the abdomen of both sexes. But in the present state of our knowledge *Eremoctenia* must stand fairly wide apart from any known form, its diagnostic characters being, as will be seen above, largely negative, that is, consisting in the absence of structures which other genera possess.

Eremoctenia progressa (Muir).

Penicillidia progressa Speiser, MS.; Muir, *Bull. Mus. Zool. Harvard*, liv, no. 11, 1912, pp. 351–2, 356–8; Pl. II, figs. 8, 10 (larva, pupa, etc., but no description of the adult).

Length of body, not including head or legs, about 2·25 mm.

Head: the remarkable shape of the capsule has been mentioned; front part of vertex rather densely clothed with stoutish bristles, which

extend also down the margins of the cheeks; palpi bearing very long stout bristles at the apex.

Thorax. Absence of ctenidia dealt with above. The chitinous part of the thorax just in front of the halter bears an irregular group of 9 or 10 bristles, not a regular series as in some forms. *Halteres* large, flattened, scale-like, with surface minutely pollinose (they recall those of *Cyclopodia sykesi*). *Ventral surface of thorax* (Fig. 2) very strongly convex from back to front, much broader than long, the exact proportions being difficult to gauge owing to the convexity: median longitudinal line marked by a fairly broad streak of darker pigment, and strongly impressed, especially at the posterior extremity: the obliquely transverse lines present in other Nycteribiidae are only discernible here with difficulty (represented in Fig. 2 by faint dotted lines), the parts being very firmly consolidated: surface-bristles very fine and short, hind margin bearing a few longer ones at the angles; surface also with two other impressions, one on either side near the lateral margin, just in front of the transverse line.

♂ Abdomen: dorsally (Fig. 1) this is very bristly indeed. *5 tergites* are visible in addition to the anal segment, but the basal one has its basal portion pale, soft, and bare; the remainder of its surface, and the entire surfaces of tergites 2 and 3, are densely covered with short, fine, sub-erect bristles: these surface-bristles are present also on tergites 4 and 5, but only near the hind-margins, the basal parts being bare. All 5 tergites have their hind-margins set with longer and shorter bristles, the long ones of tergites 2–5 being very long and stout. *Anal segment* rather short, broad at the apex, its dorsal surface bare, hind margin and sides bearing moderately long bristles, and one very long one near each hind angle.

Ventrally (Fig. 2), the *basal sternite*, though bare at its base, is otherwise rather closely covered with short bristles, those at the sides being rather longer and directed outwards: the hind margin bears no trace of a ctenidium, but only a few bristles of varying lengths, set at rather wide and irregular intervals. *Sternites 2 and 3* have their surfaces bare except for a few bristles near the hind angles: their hind margins bear bristles of varying lengths, set rather wide apart, two or more short bristles between each two long ones, the long ones near the outer angles being very long. *Sternite 4* obtusely produced in the middle behind, the apical part of the hind margin bearing a group of short, stout, blackish thorn-bristles; there is a submarginal series of short sub-erect bristles, and on either side of the thorn-bristles the margin bears bristles

of varying lengths, set rather wide apart (as on the preceding sternites), there being one very long bristle on either side near the angle; the surface of the sternite behind the submarginal series is bare. The parts of the *anal segment* visible ventrally on either side of the claspers are bare at the base, but distally have numerous erect bristles directed outwards: the *claspers* lie very wide apart, and are curved inwards and slightly dorsalwards at the apex; each bears one very long bristle near its base, and a number of short bristles, but the apical portion is bare.

♀ Abdomen (Fig. 3). *Basal tergite* of remarkable form, produced backwards with rounded margin in the middle: on the surface this middle part is bounded on either side by a line of dark brownish pigment, so that the tergite appears to consist of a nearly round median, and of two separate lateral, portions: the basal part of the median portion is soft and whitish, and bears some extremely minute rudiments of bristles, otherwise the surface is bare: the margin also is bare except in the rounded middle part, which has a series of about 14 long bristles, the median ones of which are slightly longer than the outer. *Tergite 2* sinuate and slightly produced in the middle: surface bare, except for a small median area, which bears very minute short bristles: margin furnished with a series of bristles of varying length, those in the middle close together (and the 4 nearest the middle line very long), those towards the sides wide apart. Behind tergite 2 is an expanse of bare whitish *connexivum*, posterior to which, and immediately in front of the anal segment, is a widely and bluntly triangular *chitinous area*, with a marginal series of long bristles, of which the median are longer than the lateral, and with a number of short submarginal bristles on the surface. *Anal segment* tapering considerably, its mid-dorsal portion bare (even including the hind margin), its lateral parts densely covered with moderately long erect bristles, and bearing several very long bristles near each hind angle.

Ventrally (Fig. 4), the *basal sternite* differs decidedly from that of the ♂, a difference which is exceptional among Nycteribiidae[1]: it is proportionately longer, its hind margin is more curved, the bristles of the surface are sparser, and appear to be absent in the submarginal as well as the extreme basal portion: there is no trace of ctenidium, and only a few bristles very wide apart on the median part of the margin. Posterior to this is an area of soft whitish *connexivum*, bearing a curved series of 8 very long bristles, the convexity of the curve being directed

[1] An extreme case is provided by *Cyclopodia roylei* (Westwood); see Scott, 1914*a*, p. 225.

forwards: within and behind this series the connexivum bears several irregular transverse rows of moderately long bristles, while outside the curved series the lateral portions bear extremely minute rudimentary bristles. Posterior to this connexivum are two roughly oval, convex, *chitinous areas* (cf. *Penicillidia jenynsi*); each has a dense group of bristles at its outer angle, some of which are very long, otherwise the surface of each area is almost bare, but the hind margin of each bears inwardly (*i.e.* towards the middle line of the body) 4 or 5 bristles, rather wide apart. Behind these two chitinous areas is a transverse series of bristles, rising from two slight chitinous ridges which almost meet in the middle line, and on either side of the body is a blunt protuberance bearing a group of bristles, one of which is very long: [these two bristle-bearing ridges and protuberances may possibly represent rudiments of a second pair of chitinous areas similar to those immediately in front]. *Subgenital plate*. slightly bi-lobed, the apex of each lobe bearing a group of bristles, one of which is very long; the median part of the surface also bears a number of shorter and longer bristles.

Larva: described and figured by Muir (1912, p. 356, Pl. II, fig. 8). According to him, it is about 1·6 mm. by 1·2 mm., ovoid, broader behind, of the same general form as the other Nycteribiid larvae which are known, with two pairs of spiracles, the anterior being dorso-lateral and slightly behind the middle line, and the posterior pair being postero-dorsal, quite close to the hind end of the body. At the anterior end is a small constriction bearing the mouth-opening: but Muir's description is made from full-grown larvae taken from the uteri of their parents, and as he himself states, the larvae change their shape somewhat on passing out of the uterus. Probably, therefore, this anterior constriction would disappear after the birth of the larva. No such constriction is described by Rodhain and Bequaert in the already born larvae of *Cyclopodia greeffi* (see above, p. 599). In 1908 the present writer described and figured a larva of *Penicillidia jenynsi* with a much more marked constriction near the anterior end: but in this case, as justly remarked by Rodhain and Bequaert (1915, p. 257), the constriction was doubtless due to pressure of the sides of the genital orifice of the parent, which had been killed at the moment when the larva was passing through, or being held protruding from, that orifice. The normal form of a Nycteribiid larva after birth is probably that described by Rodhain and Bequaert for *Cyclopodia greeffi*, with elliptic or ovoid contour, the anterior end being the more pointed, but not constricted. Muir's figure also shows the anterior spiracles of the larva of *Eremoctenia progressa*

as considerably further forward than are those of *Cyclopodia greeffi* or *Penicillidia jenynsi*. This may be a constant difference, or may be partly due to the slightly earlier stage of development of the larvae examined by Muir.

Puparium and pupa: these are described, and the latter is figured, by Muir (1912, pp. 357–8, Pl. II, fig. 10). His remarks have already been cited (above, p. 599) for comparison with the corresponding facts in *Cyclopodia greeffi*.

Locality. Amboyna (Dutch East Indies).

Types, ♂ and ♀, in British Museum.

The material was obtained by Mr Frederick Muir in 1908, about 30 specimens being collected from a number of individuals of *Miniopterus schreibersi*. They were submitted to Dr P. Speiser, who gave them the manuscript-name of *Penicillidia progressa*, under which name they are referred to by Muir in his paper (1912). Speiser no doubt intended to publish a description, but appears never to have done so. Meanwhile 1 ♂ and 1 ♀ (in good condition, preserved in alcohol) were given by Muir to the British Museum, where the present writer drew up a description and made figures of them. Muir (1912) published descriptions of the larva and pupa, but not of the adult, neither did he give any diagnosis for identification of the species, since he anticipated an early publication on the matter by Speiser. Therefore, since the form is a highly remarkable one, the present writer has thought it best, after re-examination of the British Museum specimens, to publish his description above. Speiser's manuscript-name "*progressa*," used by Muir in print, must be the specific name, while a new genus is erected for reasons already stated.

Genus **PENICILLIDIA**, Kolenati.

Penicillidia fletcheri Scott, *Ann. Mag. Nat. Hist.*, ser. 8, xiv, p. 214, Pl. X, figs. 1–4, 1914.

This species was described from Coimbatore, Madras, on *Pipistrellus dormeri*. It can now be recorded also from Bangalore, Mysore, collected by Rev. Father Assmuth, name of host not stated; Dr Bequaert has sent me a ♂ received from that locality, and the specimen closely agrees with the type ♂. A var. *majuscula* of this species has also been described by F. W. Edwards from West Sumatra, where it was found in numbers on "*Vespertilio* sp." (Robinson and Kloss coll.): I am informed that the description will appear in *Journ. Fed. Malay States Museums*, vol. vii.

Penicillidia fletcheri var. **pumila** Scott, *op. cit.* p. 217, Pl. X, fig. 5.

Described from Peradeniya, Ceylon, on *Pipistrellus abramus*. It can now be recorded from Khandala, Bombay Presidency; Rev. Father Assmuth coll., name of host not stated. Dr Bequaert has sent me two ♀ received from that place, which agree with the type in all particulars, except that they both have the two groups of bristles on the small basal tergite much longer. In his letter Dr Bequaert remarks that he is inclined to give var. *pumila* rank as a separate species, since the differences between it and typical *fletcheri* seem marked and constant. Since it has now been found in India, it cannot be an exclusively Ceylonese race of *fletcheri*.

Genus **CYCLOPODIA.**

DIVISION INTO SUBGENERA AND ERECTION OF A NEW GENUS.

In describing *Cyclopodia roylei* (Westwood) I stated (1914*a*, p. 225, footnote) that it differs in some ways from all other *Cyclopodiae* known to me. Its separation in a distinct subgenus, *Paracyclopodia*, is proposed below. A distinct new genus, *Tripselia*, is also proposed for one or more species described some time since.

A genus *Basilia* was described by A. de Miranda Ribeiro (*Arch. Mus. Rio Janeiro*, XII, pp. 175–9, Pl. I, 1903) for a South American Nycteribiid[1] characterised by the presence of eyes composed of more than one facet, as in *Cyclopodia*, but also by the *absence* of tibial rings. The same writer later described a second genus[2], *Pseudelytromyia*, which however is regarded by Speiser (1908, p. 437) as a synonym of *Basilia*. Speiser has recognised *Basilia* as distinct, and has also referred to it certain Old-World species. But Brèthes, in describing a new *Cyclopodia* from Chili (*Boletin del Museo Nacional de Chile*, pp. 1–4 and Figs., 1913), considers *Basilia* to be a synonym of *Cyclopodia*. Presumably he imagines that the tibial rings are present, but have been overlooked. This is possible, as the rings are not always easy to see, especially if the specimens are not very mature and the chitin consequently is pale. I have seen no representative of *Basilia*, and shall assume for the present that it is distinct. *Cyclopodia*, *Tripselia*, and *Basilia* may then be separated as follows:

(A) Eyes present, composed of more than one facet. Tibiae 3-ringed...*Cyclopodia*.

[1] *Basilia ferruginea*, on *Vespertilio aurantius*.

[2] On *Atalapha frantzii* Peters. (Vespertilionidae): *Pseudelytromyia* was described *op. cit.* XIV, pp. 233–5, Pl. XXIII–IV, 1907.

(B) Eyes absent. Tibiae 3-ringed...*Tripselia*.

(C) Eyes present, composed of more than one facet. Tibiae not ringed...*Basilia*.

One may proceed to a more complete diagnosis of the two former.

Genus **CYCLOPODIA**, Kolenati.

Tibiae 3-ringed. *Eyes* composed of more than one facet on a dark pigmented ground.

Subgenus **Cyclopodia**, s. str.

Head broad, somewhat compressed in the horizontal, but not at all in the vertical, plane, its dorsal surface between the eyes only slightly arched. *Anterior coxae* much elongated. *Hosts*, so far as known, all frugivorous (Pteropidae).

Type of subgenus: *Cyclopodia sykesi* (Westwood).

Other species actually examined by me which conform to the above diagnosis are: *C. horsfieldi* de Meij., *C. albertisi* Rondani, *C. greeffi* Karsch (= *rubiginosa* Bigot[1]), *C. oxycephala* Bigot, *C. ferrarii* Rondani. In all these each *eye* appears to consist of two facets, and this number may be constant for the whole subgenus (or even for the genus). In all the above-named species except *C. ferrarii* the *tibiae* are nearly cylindrical in section, but in *C. ferrarii* they are rather flattened laterally, as in *C.* (*Paracyclopodia*) *roylei*, but *not* broadened as in subgenus *Listropodia* of *Nycteribia*. In most, if not all, of the above-named members of the subgenus *Cyclopodia*, the hollow containing the ♂ intromittent organ in the ventral side of the anal segment, over the claspers, does not extend as far forward as the apex of the claspers; while in *C.* (*Paracyclopodia*) *roylei*, and in certain other genera, it does extend so far forward. The *halteres* differ: in a number of the large species (*e.g. C. sykesi*, *C. oxycephala*) they are large, flattened and scale-like, and minutely pollinose: but in *C. ferrarii* and *C. greeffi* they are very small, with slender pedicel and knobbed apex.

Cyclopodia horsfieldi de Meijere, *Tijdschr. v. Ent.* xlii, 1899, p. 153.

A hitherto unpublished record for this species is that of a number of ♂ and ♀ from the Philippine Islands: La Carlota, Occidental Negros, taken from *Pteropus philippinensis*, 8–9, ix, 1911: sent by Dr H. B.

[1] See footnote, p. 596.

Mitzmain to the Quick Laboratory, Cambridge: the material is now at Cambridge and in Brit. Mus.[1]

The species has previously been recorded from Java, Sumatra, and Engano, without record of host; and (by Speiser, 1903) from the Malay Peninsula, from *Pteropus vampyrus* (Linn.).

Subgenus **PARACYCLOPODIA**, nov.

Head very narrow, strongly compressed in the vertical plane, strongly arched dorsally between the eyes; its form is like that found in *Nycteribia* and other genera. *Anterior coxae* not much elongated. *Hosts*, so far as known, insectivorous and carnivorous (species of Vespertilionidae and Nycteridae).

Type of subgenus: *Cyclopodia* (*Paracyclopodia*) *roylei* (Westwood); Scott, 1908, p. 368, 1914*a*, p. 224 (= *Nycteribia chlamydophora* Speiser, 1903).

I am not acquainted with any other species of the subgenus. In *C. roylei* the *tibiae* are somewhat flattened laterally, but not broadened, resembling in form those of *C.* (*s. str.*) *ferrarii*, referred to above. The *eyes* are dark-pigmented, and consist each of at least two facets, as in *Cyclopodia* s. str. The front *coxae* are not elongated like those of *Cyclopodia* s. str., but are no more elongated than in some species of other genera. The hollow above the ♂ claspers extends as far forward as the apex of the claspers; contrast *Cyclopodia* s. str. *Halteres* small and erect, with slender pedicel and knobbed apex.

C. roylei appears to be not uncommon and widely distributed in India, Ceylon, and the Malay Peninsula. For a list of localities and hosts, see my paper (1914*a*); an additional record is: 1 ♂, 2 ♀, pale and small, from *Scotophilus wroughtoni*, at Helwak, near Satara, Western Ghâts, India, 4, v, 1900: N. C. Rothschild don.

Genus **TRIPSELIA**, gen. nov.

(*Tripselia*, Speiser MS., in litt. 1908, from ψέλιον, an armlet.)

Tibiae 3-ringed, as in *Cyclopodia*. *Eyes* quite absent. *Head* narrow, laterally compressed. *Anterior coxae* not much elongated.

Type of genus: *Tripselia fryeri* (Scott); described as *Nycteribia* (*Acrocholidia*) *fryeri* Scott, *Trans. Linn. Soc. London*, ser. 2, *Zool.*, XVII, p. 163, 1914.

[1] Since the above was printed I have received through Dr Bequaert 2 ♂ and 1 ♀ of *C. horsfieldi* from another island of the Philippines: Porto Galera, Mindoro, on large fruit-bats.

The *head* resembles in form that of *Cyclopodia* (*Paracyclopodia*) *roylei* and of many species of *Nycteribia*, etc. The *legs* are long and very slender, the tibiae not appreciably flattened. The *front coxae* are no more elongated than in many species of *Penicillidia*, etc. The hollow above the ♂ claspers extends as far forward as the apex of the claspers. *Halteres* small and erect, with knobbed apex and slender pedicel.

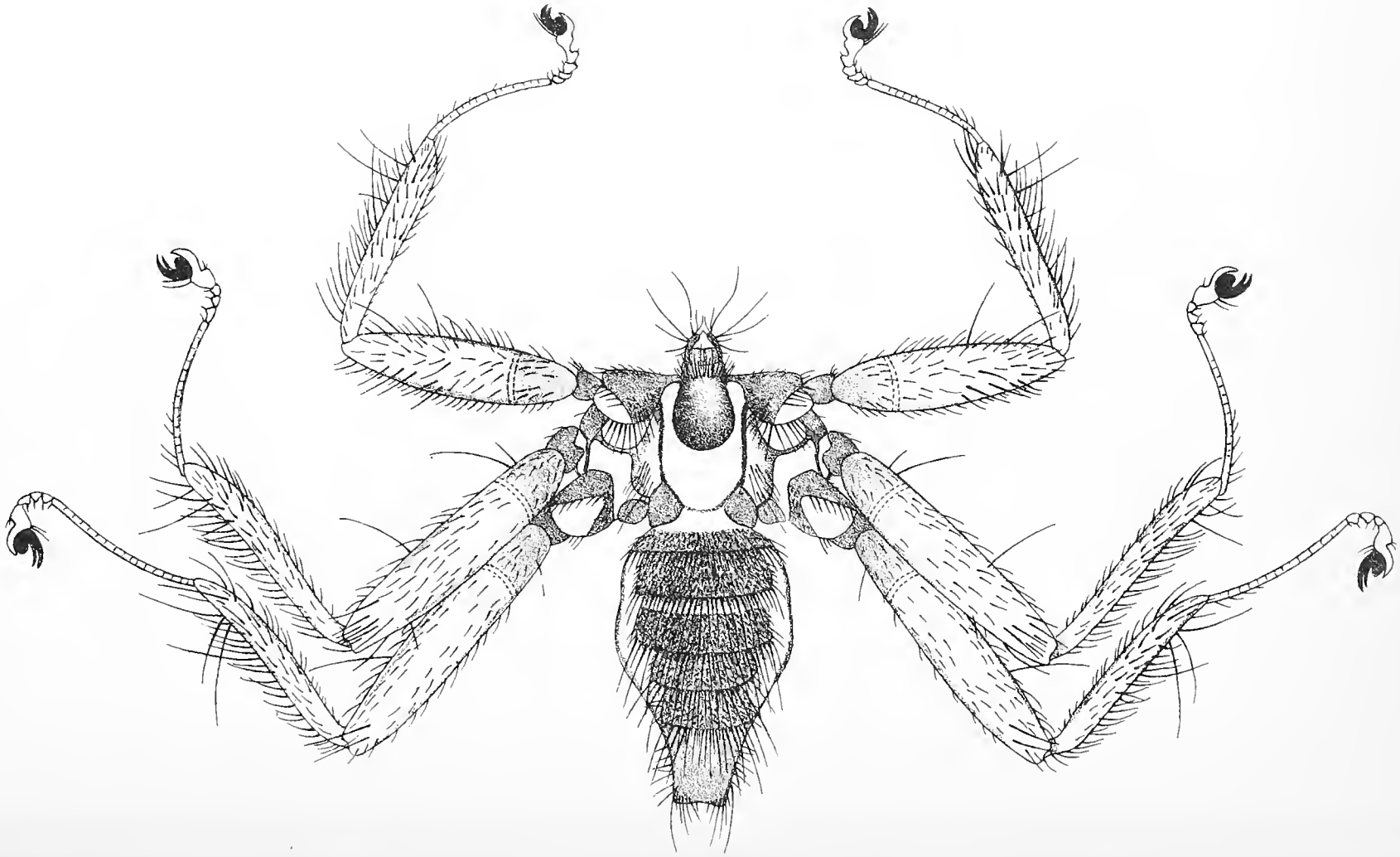
T. fryeri was described from Assumption Island (N. of Madagascar) and from Labuan. Dr Bequaert has since collected 4 ♀ in the Belgian Congo, at Medje, and has called my attention to the presence of the tibial rings, the overlooking of which caused me to place the species in a wrong genus. I have examined one of his ♀ side by side with the series from Assumption, and am convinced of their identity, and he states that the other 3 examples also agree closely with my description.

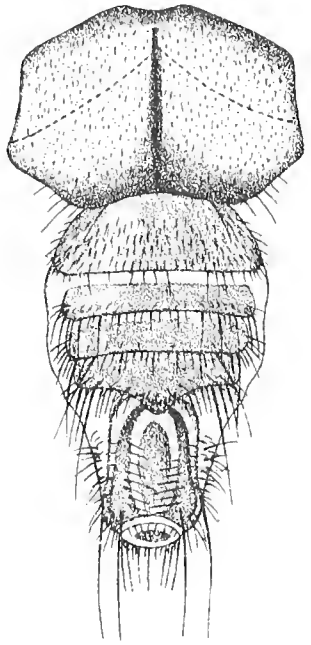
Possibly *Cyclopodia amiculata* Speiser (*Rec. Ind. Mus.* I, 1907, p. 296), which I have not seen, is also a *Tripselia*. Though Speiser placed it in *Cyclopodia* on account of its 3-ringed tibiae, he wrote to me in 1908 that on re-examination it proved to have either no eyes at all, or only a single unpigmented lens on either side of the head—probably the former. In either case he considered it could not remain in *Cyclopodia*, and proposed the generic name *Tripselia*, which I now adopt. If *amiculata* has no eyes at all, it is certainly a *Tripselia* (*sensu meo*). But if it has single unpigmented lenses like those of *Penicillidia* and *Eucampsipodia*, it may necessitate the erection of yet another genus. Furthermore, if *amiculata* is a *Tripselia*, it is possible that my *T. fryeri* is identical with it. With his letter Speiser sent rough sketches of the abdomen of ♂ *amiculata*, not unlike the abdomen of *fryeri* ♂. His published description of the ♀ was very short and only intended as preliminary, but it mentions characters which make me suspect the identity of *fryeri* with *amiculata*.

[Since the above was written I have received through Dr Bequaert 1 ♂ and 1 ♀ from Sumatra, belonging to a form which very closely resembles *T. fryeri* except in the following particulars: size smaller; legs noticeably shorter, especially femora, and both femora and tibiae stouter; ventral hind margin of thorax in both sexes fringed with long bristles (absent in typical *fryeri*), three on each side of the middle line, with shorter ones between them. Detailed consideration of this form, stated to be from *Pipistrellus* sp., must be deferred.]

Hosts of *Tripselia fryeri* are: *Taphozous mauritianus* (Emballonuridae), Assumption Island; *Saccolaimus* (= *Taphozous*) *saccolaimus*, Labuan; *Saccolaimus* (= *Taphozous*) *peli*, Belgian Congo.

NYCTERIBIIDÆ.





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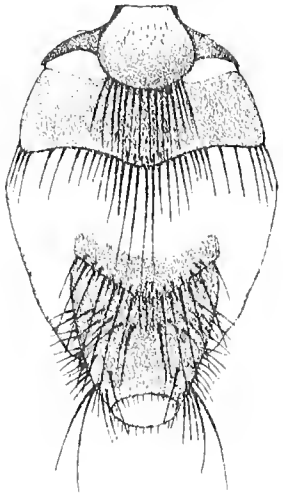

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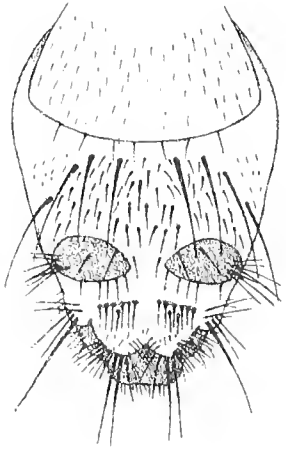

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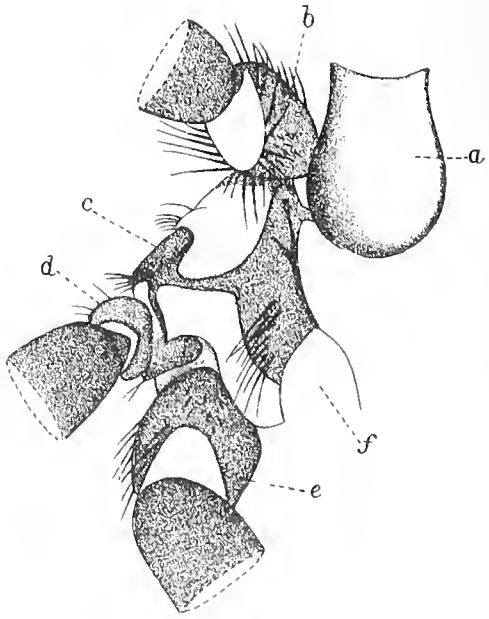

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H. Scott del

Eremoctenia progressa (Muir).

Speiser's *amiculata* was described from Calcutta, from *Taphozous longimanus*.

Genus **EUCAMPSIPODIA**, Kolenati.

Eucampsipodia hyrtli (Kolenati), *Horae Soc. ent. Ross.* II, 1863, p. 78, Pl. XII, figs. 26 *d*, *e* (♀), Pl. XIV, figs. 26 *a–c* (♂); Scott, *Ann. Mag. Nat. Hist.* ser. 8, XIV, pp. 213, 228–230, Pl. XII, figs. 18, 19, 1914.

A correction. In my paper (*l.c.*) I twice stated that Kolenati only figured the ♂ sex of this species. This was erroneous, for he also figured the ♀. I was misled by the fact that his figures of the two sexes are on two different plates between which a third plate intervenes.

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EXPLANATION OF PLATE XXIV.

Eremoctenia progressa (Muir).

Fig. 1. ♂, dorsal view, × circa 27.

Fig. 2. ♂, ventral view of thorax and abdomen.

Fig. 3. ♀, dorsal view of abdomen.

Fig. 4. ♀, ventral view of abdomen.

Fig. 5. Left side of thorax and bases of legs from above, to larger scale, to show complete absence of thoracic ctenidium: *a*, outline of head; *b*, front coxa; *c*, middle coxa; *d*, middle trochanter; *e*, hind coxa; *f*, halter.

Note.—All the figures were made with the help of a drawing-apparatus, from specimens lying in alcohol.

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